

Potential of Safflower (*Carthamus tinctorius* L.) for Phytoremediation of Soils Contaminated with Heavy Metals

Violina R. Angelova, Vanja I. Akova, Stefan V. Krustev, Krasimir I. Ivanov

Abstract—A field study was conducted to evaluate the efficacy of safflower plant for phytoremediation of contaminated soils. The experiment was performed on an agricultural fields contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. Field experiments with randomized complete block design with five treatments (control, compost amendments added at 20 and 40 t/daa, and vermicompost amendments added at 20 and 40 t/daa) were carried out. The quality of safflower seeds and oil (heavy metals and fatty acid composition) were determined. Tested organic amendments significantly influenced the chemical composition of safflower seeds and oil. The compost and vermicompost treatments significantly reduced heavy metals concentration in safflower seeds and oils, but the effect differed among them. Addition of vermicompost and compost leads to an increase in the content of palmitic acid and linoleic acid, and a decrease in the stearic and oleic acids compared with the control. A significant increase in the quantity of saturated acids was observed in the variants with 20 t/daa of compost and 20 t/daa of vermicompost (9.1 and 8.9% relative to the control). Safflower is a plant which is tolerant to heavy metals and can be successfully used in the phytoremediation of heavy metal contaminated soils. The processing of seeds to oil and using the obtained oil for nutritional purposes will greatly reduce the cost of phytoremediation.

Keywords—Heavy metals, organic amendments, phytoremediation, safflower.

I. INTRODUCTION

HEAVY metal contamination of agricultural soils is a worldwide problem. The remediation of metal contaminated sites often involves expensive and environmentally invasive and civil engineering based practices [1]. A range of technologies such as fixation, leaching, soil excavation, and landfill of the top contaminated soil ex situ have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution [2] or adverse effect on biological activities, soil structure, and fertility [3]. 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], [4]. 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 [5], [6]. It is best applied at the sites with shallow contamination of organic, nutrient or metal pollutants [7]. The use of crop plants for phytoremediation of contaminated soils has the advantages of their high biomass production and adaptive capacity to variable environments [8]-[13]. However, to succeed they must be tolerant to the contaminants and be capable of accumulating significant concentrations of heavy metals in their tissues. Additionally, crops could make the long time-periods for decontamination more acceptable, economically and environmentally. If the contaminated biomass may be further proceed for added value products (not only concentrated on deposits of hazardous wastes), then such fact represents an improvement of economic efficiency of phytoremediation technology. Industrial plants, i.e. energy crops or crops for bio-diesel production, are therefore the prime candidates as plants for phytoremediation. The use of energy and/or bio-diesel crops as plants for phytoremediation would give contaminated soil a productive value and decrease remediation costs.

Safflower (*Carthamus tinctorius* L., *Asteraceae*) is an annual plant originally grown for its flowers, which were used in making red and yellow dyes for clothing and food preparation. Today, safflower it is primarily cultivated for its oil, which is used for food and industrial purposes. Safflower can be used as animals feeds, birds feed and in small quantity for ruminants feed as whole seeds or meals [14]. The seed contains nearly 35–50% oil, 15–20% protein, and 35–45% hull fraction [15], [16]. There are linoleic and oleic types of safflower varieties. The oil in linoleic varieties contains about 70–80% linoleic acid and is used for edible oil products such as salad oils and soft margarines. The oil of oleic varieties contains about 80% oleic acid and serves as a heat-stable cooking oil, or as a drying or semi-drying oil for paints and other surface coatings [17].

In our previous studies, it was found that the safflower may be grown on the contaminated soils with heavy metals [18]. Addition of organic matter amendments, such as compost, and vermicompost in soil leads to immobilization of heavy metals and soil amelioration of contaminated soils [19], [20]. Organic amendments are able to improve soil physical, chemical and biological properties by: (i) raising the pH, (ii) increasing the organic matter content, (iii) adding essential nutrients for plant

V. R. Angelova is with the Agricultural University – Plovdiv Bulgaria (corresponding author to provide phone: 357 888 578126; fax: 357-32-633-157; e-mail: vileriz@abv.bg).

V. I. Akova, S. V. Krustev, and K. I. Ivanov are with the Agricultural University – Plovdiv Bulgaria (PhD student) (e-mail: vanjal@abv.bg, krust@abv.bg, kivanov1@abv.bg).

growth, (iv) increasing the water holding capacity, and (v) modifying heavy metals bioavailability [20]-[22].

The aim of this experiment was to compare the effect of organic soil amendments (compost and vermicompost) applied to the soil on the quality of safflower seeds and oil (heavy metals and fatty acid composition), as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

II. MATERIAL AND METHODS

The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The field experimental was a randomized complete block design containing five treatments and four replications (20 plots): 1 - introduction of 20 t/daa of vermicompost to the soil, 2 - introduction of 40 t/daa of vermicompost to the soil, 3 - introduction of 20 t/daa of compost to the soil, 4 - introduction of 40 t/daa of compost to the soil, 5 - control variant.

Characteristics of soils and organic amendments are shown in Table I. The soils used in this experiment were slightly acidic, with moderate content of organic matter and essential nutrients (N, P and K) (Table I). The pseudo-total content of Zn, Pb and Cd is high (1430.7 mg/kg Zn, 876.5 mg/kg Pb and 31.4 mg/kg Cd, respectively) and exceeds the maximum permissible concentrations (320 mg/kg Zn, 100 mg/kg Pb, 2.0 mg/kg Cd).

TABLE I
CHARACTERIZATION OF THE SOIL AND THE ORGANIC AMENDMENTS USED IN THE EXPERIMENT

Parameter	Soil	Compost	Vermicompost
pH	6.5	6.9	7.5
EC, dS/m	0.2	0.2	2.2
Organic C,%	2.22	40.50	21.43
N Kjeldal,%	0.24	2.22	1.57
C/N	9.25	18.24	13.65
Pseudo-total P, mg/kg	642	12653.9	10210.8
Pseudo-total K, mg/kg	5517.5	6081.7	10495.1
Pseudo-total Pb, mg/kg	876.5	12.02	32.25
Pseudo-total Zn, mg/kg	1430.7	170.77	270.3
Pseudo-total Cd, mg/kg	31.4	0.192	0.686

The test plant was safflower (*Carthamus tinctorius* L.). Safflower seeds were sown in each plot; between row and within row distances were 60 and 20 cm, respectively. Each hole was 5–6 cm deep, containing 3 seeds. After safflower had grown for 15 days, the safflower was thinned to one plant per hole. Upon reaching commercial ripeness, the safflower plants were gathered and the seeds were ground in a Wiley mill with the 1 mm mesh sieve. The oil from ground safflower seed was derived under laboratory conditions through an extraction method with Soxhlet's apparatus. The contents of heavy metals in safflower seeds and oils were determined by the method of the dry mineralization. To determine the chemical composition in the samples, inductively coupled emission spectrometry (Jobin Yvon Emission - JY 38 S, France) was used. Nitrogen determinations were made on the ground

extracted seed by the Kjeldahl procedure [23], and crude protein content was calculated by the factor $N \times 5.3$.

For fatty acid composition analysis, the preparation of the methyl esters of the fatty acids was performed following the procedures described in [24]. The analysis of the methyl esters of the fatty acids was carried out by gas chromatography according to [25]. The individual composition of fatty acid was determined by comparing the retention times obtained from the chromatogram of the analyzed mixture with the times of witnesses - methyl esters of fatty acids. Individual fatty acid composition was determined by comparing the retention times obtained from the chromatogram of the test mixture with the times of witnesses - methyl esters of fatty acids. The quantitative composition of the fatty acids in the same mixture is determined by the percentage ratio between the areas of the individual peaks in the chromatogram.

Statistical analyses were conducted with Statistica v. 7.0.

III. RESULTS AND DISCUSSION

Humic acids from organic amendments tend to form complexes that are different for each metal and also depend on soil conditions such as pH, cation exchange capacity and clay mineral fraction [26]. Organic matter not only forms complexes with these metals but it also retains them in exchangeable forms, affecting each metal differently. Some metals are bound and rendered unavailable while others are bound and readily available [27]. The results of the previous study [18] indicated that enrichment of soil with organic matter could reduce the content of bioavailable metal species as a result of complexation of free ions of heavy metals. This is indicative of heavy metals immobilisation by humic substances from compost and vermicompost application. Obtained results appear that verify the function of humic acid in improving phytoremediation efficiency of soils contaminated with heavy metals; and potential environmental availability of metals may be controlled by soil organic amendments.

A. Content of Heavy Metals in Seeds

Fig. 1 presents the results obtained for the content of heavy metals the reproductive organs of the study oilseed crop. The heavy metal content in the seeds is significantly lower compared to the root system and the aboveground biomass of the plants. Their accumulation into the safflower seeds is likely to occur through the vascular tissue system of the plant. The content of Pb and Zn in the seeds of the control is 3.57 mg/kg and 133.5 mg/kg and does not reach the critical value of 30 mg/kg for Pb and 300 mg/kg for Zn [28]. Cd accumulates in the seeds (2.9 mg/kg) at levels significantly above the recommended maximum levels tolerated by animals (0.5 mg Cd /kg [29] and the recommended values for food (1 mg/kg) [28].

Introduction of compost and vermicompost significantly affect the uptake of heavy metals (Pb, Zn and Cd) of the safflower plants. Introduction of compost leads to a lower content of heavy metals, as with most elements with the increase of the amount of the additive, the decrease is greater.

With the introduction of vermicompost, the tendency is the same. The influence of organic ameliorants on the accumulation of heavy metals in the safflower seeds depends essentially on their quantity.

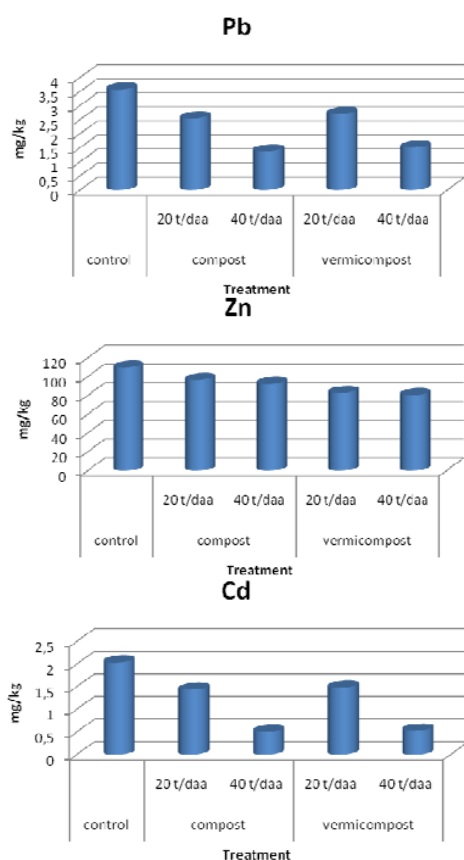


Fig. 1 Effect of different organic amendments (compost and vermicompost) applications to accumulation of heavy metals in reproductive organs of safflower

The reduction of the heavy metal content in the seeds compared to the control is strongly expressed, as in the compost variant the Pb content decreases from 3.6 mg/kg to 1.4 mg/kg, while in the vermicompost variant - to 1.5 mg/kg and these values are lower than the maximum feed concentration (30 mg/kg). Relationship has been found between the amount of the imported ameliorant and the Pb content in the safflower seeds. With the increasing amount of the ameliorant, the Pb content decreases. The results for cadmium are similar. The reduction of the Cd content in the seeds compared to the control is strongly expressed, as in the variants with the introduction of 40 t/daa of compost and 40 t/daa of vermicompost, the Cd content in the seeds decreases from 2.9 mg/kg to 0.5 mg/kg and these values are within the limits for forage [28].

The content of Zn in the safflower seeds after the introduction of organic ameliorants reduces. This decrease is more pronounced after the introduction of vermicompost (from 109.6 mg/kg to 84.5 mg/kg), while with the introduction

of compost the decrease is significantly less - to 92.4 mg/kg. No correlation has been established between the amount of the imported organic ameliorants and the content of Zn in the safflower seeds. However, the introduction of organic additives lowers the amount of zinc to levels that are lower than the maximum levels for forage.

B. Content of Heavy Metals in Safflower Oil

The results obtained show that the main part of the heavy metals contained in the seeds, during processing does not go into the oil, due to which their content is significantly lower than the received MAC /maximum allowable concentrations/.

The content of Pb in safflower oil reaches up to 0.21 mg/kg. MAC for Pb in oil of vegetable origin is 0.1 mg/kg. These results strongly suggest that the major part of Pb, contained in the seeds of safflower, does not pass into the oil obtained, however its content in the oil is slightly higher than the MAC, and it cannot be used for food purposes.

The content of Cd is below the limits of the quantitative measurement with the method used. Under the current standard, the content of Cd should not exceed 0.05 mg/kg. Although the seeds contain Cd, during their processing it does not pass into the oil and it can be used for food purposes.

MAC for the content of Zn in the vegetable fats is 10 mg/kg. These results strongly suggest that the major part of Zn, contained in the seeds of safflower, does not pass into the oil obtained, however its content in the oil is slightly higher than the MAC.

The importation of organic meliorants significantly reduces the content of heavy metals in the oil. Fig. 2 shows the results of the impact of the organic meliorants on the chemical composition of the oil. Their influence on the content of Pb and Zn in the oil essentially depends on their quantity. The increase in the amount of supplement imported (40 t/decare compost and 40 t/decare vermicompost) reduces the content of Pb in the oil respectively to 0.09 mg/kg and these concentrations are lower than the maximum allowable concentrations of oil of vegetable origin (0.1 mg/kg). The importation of compost and vermicompost reduces the zinc content in the oil, and this decrease is more strongly shown in the importation of 20t/decare of compost and 20t/decare vermicompost (respectively to 5.9 mg/kg and 2.98 mg/kg). In all options, however, Zn content in the oil is lower than the maximum allowable concentrations of oil of vegetable origin (10 mg/kg).

The importation of organic meliorants is particularly effective in reducing the content of lead and zinc in safflower oil below the limit values (respectively 0.1 mg/kg Pb and 10.0 mg/kg Zn), which makes it suitable for use for food purposes.

The content of Cd in safflower oil with all options is below the limits of the quantitative measurement with the method used both in the control and in the variants with adding organic meliorants. The importation of organic meliorants has no significant effect on the content of cadmium in the oil and it can be used for food purposes.

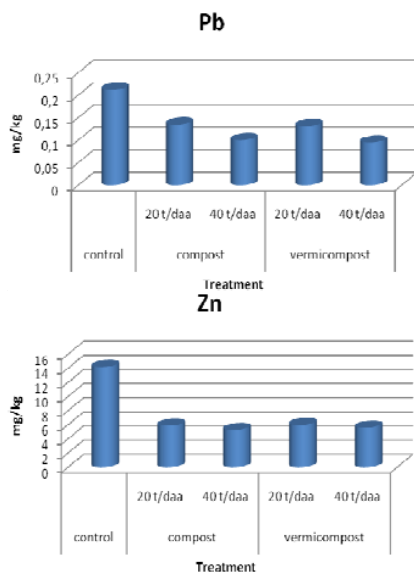


Fig. 2 Effect of different organic amendments (compost and vermicompost) applications to accumulation of heavy metals in safflower oil

C. Fatty Acid Composition of Safflower Oil

Safflower oil is considered one of the best-quality vegetable oil containing mainly palmitic, stearic, oleic and linoleic acids [30].

In the fatty acid composition of the tested oil obtained from the extraction of safflower seeds from the control predominant are the unsaturated fatty acids and their amount respectively reaches up to 91.6%.

In the composition of the oil dominant is the linoleic acid (C18:2, 76.22%), followed by the oleic acid (C18:1, 14.93%), which attributes it to the linoleic type of oils. Standard safflower oil contains 5-8% of palmitic acid, 2-3% of stearic acid, 8-20% of oleic acid and 68-83% of linoleic acid [31]. The presence of linolenic (C18:3, 0.07%), gadoleic (C20:1, 0.15%) and erucic (C22:1, 0.20%) acids has also been determined.

Safflower oil is characterized by the lowest level of saturated fatty acids among the cultured oilseed crops [32].

Of the saturated fatty acids, palmitic acid (C16: 0) predominates in an amount of 6.04%, followed by stearic acid (1.91%). The oil also contains lauric (C12:0, 0.09%) and arachidonic (C 20:0, 0.32%) acids. The content of the saturated fatty acids in the control reaches up to 8.4%.

The composition of the fatty acids in the oil is the main factor that determines the use of oil for food, industrial or pharmaceutical purposes, as the variety, climate and region of production have a significant influence [33].

According to [3] the quantity and quality of fats are strongly dependent on the agro-meteorological and climatic conditions and the varietal origin. Studies by [34] have also shown that the temperature, humidity, light, and nutrients influence significantly the quantity and quality of the formed fat. Oil with higher fat content is obtained with irrigating, moderately humid conditions and optimum temperature. The fertilization with nitrogen and phosphorus fertilizers

contribute to the significantly increase in the amount of fat and improve their quality.

In the field test carried out by us, the above factors affect the quality and quantity of the fat formed. The imported soil organic meliorants, however, also have significant influence on the fatty acid composition. The oil content in the different varieties of safflower can vary from 20% to 40%. Oil content in safflower seed is a very important indicator from an economic point of view and it is considered one of the most important factors affecting the introduction of safflower in new areas of the world [35]. According to the literature data the content of oil in safflower varies between 23.86-40.33% [36], 26.72-35.78% [37], 26.3-28.5% [38] and 31.3-36.3% [39].

The data presented in Table II show the effect of organic soil meliorants on the fat content of the seeds and the fatty acid composition of the oil. Similar to the results obtained for the impact of organic meliorants on the heavy metal content in the seeds, they also influence the amount of oil.

Both the type and the quantity of the used meliorant are influential. The increase in the fat content of the seeds compared to the control is found in the variants with vermicompost, as in the variant with 20 t/decare vermicompost reaches up to 28.59%. The importation of compost to the soil lowers the fat content of the seeds. By increasing the amount of additive the fat content of the seeds decreases significantly. In the variant with 20 t/decare of compost a slight decrease is shown, while in the version with 40 t/decare of compost the reduction reaches up to 20.86% (Table II).

According to [40] the addition of vermicompost can increase the fat content of the seeds. It is believed that the vermicompost not only increases the nitrogen content of the soil, but it also contains different substances that stimulate the growth, including idol acetic acid, gibberellin and vitamins B [41].

According to [42] there is a relation between the oil content and the amount of protein in the seeds, as the lower content of oil is associated with a higher content of proteins in the seeds. Such dependence is also established in our tests.

The influence of the organic meliorants on the oil content and the protein content of seeds is presented in Table II, which shows that the content of oil and proteins in the seeds of the safflower is influenced by the meliorants used. The importation of compost results in decreased content of oil and protein in the seeds compared to the control. The importation of vermicompost does not significantly affect the fat content of the seeds and the protein content.

TABLE II
THE FATTY ACID COMPOSITION OF SAFFLOWER OIL (EXPRESSED AS % OF TOTAL FATTY ACID COMPOSITION)

Parameter	Control	Compost		Vermicompost		Codex Standard
		20 t/daa	40 t/daa	20 t/daa	40 t/daa	
Oil content, %	27.15	24.87	20.86	28.59	27.35	
Protein content, %	15.48	12.43	12.93	15.08	15.61	
Fatty acids						
Saturated						
Lauric acid C 12:0	0.09	0.09	0.10	0.10	0.10	nd
Palmitic acid C 16:0	6.04	6.83	6.54	6.66	6.57	5.3 – 8.0
Stearic acid C 18:0	1.91	1.88	1.73	1.90	1.87	1.9 – 2.9
Arachidic acid C 20:0	0.32	0.30	0.33	0.25	0.28	0.2 -0.4
Unsaturated						
Palmitoleic acid C 16:1	0.07	0.08	0.1	nd	nd	nd – 0.2
Oleic acid C 18:1	14.93	14.03	14.45	14.44	14.48	8.4 – 21.3
Linoleic acid C 18:2	76.22	76.37	76.46	76.64	76.70	67.8 -83.2
α - Linolenic C 18:3	0.07	0.08	0.08	nd	nd	nd - 0.1
Eicosenoic acid C 20:1	0.15	0.13	nd	nd	nd	0.1 – 0.3
Erucic acid C 22:1	0.20	0.20	0.21	nd	nd	nd -1.0
Saturated: Unsaturated	8.0: 92.0	8.8: 91.2	8.5: 91.5	8.7: 91.3	8.5: 91.5	
Unsaturated/saturated	10.937	9.966	10.470	10.222	10.338	
C18:1/C18:2	0.196	0.184	0.189	0.188	0.189	

ND- Non Detected

The research [43], however, does not establish such a link in the safflower seeds. The increase of the temperature leads to an increase in the availability of nitrogen in soil and an increased absorption of safflower is observed. This additional nitrogen can compete with the carbon chains (backbones) in the developing seeds and possibly turn the available carbon in proteins rather than fat. As a result, there is an increased content of the protein with the increase of the temperature. According to the authors, however, the changes do not reduce the fat content of the seeds, and are at the expense of the coating of the seeds or other component contained in them.

The results obtained for the fatty acid composition of the oil show that no significant differences are observed between the control and the variants with imported organic meliorants ($P < 0.01$). In all tested oils the following prevail: linoleic (76.22 - 76.70%), followed by oleic (14.03-14.93%), palmitic (6.04-6.83%), and stearic (1.73-1.91%) acids, and these fatty acids together comprise from 99.1 to 99.6% of the total amount of fatty acids in all the analyzed variants (Table II).

Minimum amounts of lauric (C12:0), palmitoleic (C16:1), arachidonic (C20:0), linoleic (C18:3), gadoleic (C20:1) and erucic (C22:1) acids are contained in the oil, as their values do not exceed 0.67% of the total fatty acids (Table II). It has been found that the arachidonic acid in the oil ranges from 1.2 to 3.6% [44]. Our results for the arachidonic acid are considerably lower than those established by [44].

The nutritional quality of safflower, as in most fats and oils, is determined by the fatty acid composition and in particular by the amount of oleic and linoleic acid, which makes it attractive for food purposes. Our results indicate that the unsaturated fatty acids (linoleic and oleic) represent about 90.69-91.44% of the total fatty acids in all samples analyzed (Table II).

Organic soil meliorants influence also the content of saturated and unsaturated acids in the oil. The data in the

Table II show that the used organic meliorants have negligible impact on the amount of linoleic acid in safflower oil. Linoleic acid is the predominant fatty acid which ranges from 76.22 to 76.79% of the total fatty acids in the different variants. The increase in the content of linoleic acid in comparison to the control is less pronounced, as in the variant with 40 t/decare of vermicompost it reaches 76.70%. Similar results were obtained by [45], who found increase of the content of linoleic acid in sunflower after using the combination of poultry manure and NPK fertilizer.

The addition of 20t/decare and 40 t/decare of compost also results in a minor increase in the content of linoleic acid. There is a correlation between the content of linoleic acid and the amount of imported meliorant – the increase of meliorant results in the increase of the content of linoleic acid. Linoleic acid lowers the blood cholesterol (especially LDL – cholesterine), improves the rhythm of the heart, regulates the hormone metabolism, has an antagonistic action to cholesterol and is important in the prevention and treatment of atherosclerosis and other cardiovascular diseases. On the other hand, linoleic acid is easily oxidized and reduces the quality of food products during storage, so the reduction of its content makes the oil more resistant to rancidity.

The organic meliorants used influence the content of oleic acid. It is known that the content of oleic acid decreases as a result of water stress caused by dryness of the plants in the phase of formation and dispensing of the seed. Petcu [46] examines the impact of drought and establishes a significant negative correlation between the content of oleic acid and the drought. In the experiment conducted by us the plants are watered regularly. The changes in the content of oleic acid are due solely to the influence of organic meliorants. A reduction has been found in the content of oleic acid in comparison to the control, as in the variant with 20 t/decare of compost it reaches up to 14.05%. Our results do not confirm the findings

of [47], according to whom the addition of an organic fertilizer leads to an increase in the content of oleic acid.

There was no correlation between the content of oleic acid and the amount of imported vermicompost. With the increase of the amount of vermicompost, the oleic acid content remains unchanged (Table II).

It has been found that there is a strong negative correlation between the content of linoleic and oleic acid, as the increase of the one leads to the reduction of the other [48], [49]. Our results do not fully confirm this correlation. The importation of organic meliorants leads to changes in the content of both acids and the relation between linoleic and oleic acid in safflower oil is not clearly pronounced.

The highest content of linoleic acid (76.70%) was found in variants with the importation of 40 t/decare of vermicompost, while the lowest is the content of oleic acid (14.03%) in the variant with the importation of 20 t/decare of compost (Table II). Our results do not conform to those established by [50], according to which the highest linoleic acid content corresponds to the lowest content of oleic acid.

Between the content of fatty acids and linoleic acid in safflower oil, a negative correlation was established with that of the linoleic acid [51].

Linolenic acid (C18:3) is contained in the control (0.07%) and in the variants with importation of compost (0.08%), while in variants with vermicompost it was not found.

Our results are in accordance with those of [50], who found that linoleic acid is present in less than 0.3%, as in part of the seed samples analyzed by them it is even absent. According to [52] it is possible to increase the level of linoleic acid and at the same time to reduce the linolenic acid. There is a negative correlation found between linolenic and linoleic acids.

The organic meliorants used influence the composition of saturated acids. The addition of compost and vermicompost leads to a slight increase in the amount of lauric acid.

Palmitic acid is the major saturated fatty acid, followed by stearic and these fatty acids together constitute about 7.95-8.56% of the total content of fatty acids in the oil. The content of palmitic acid is increased by importation of organic meliorants (compost and vermicompost) (Table II).

The amount of imported meliorant is also influential. The palmitic acid content ranges between 6.04-6.83% in separate variants, as the highest is the content of palmitic acid in the importation of 20 t/decare of compost. There were no significant differences in the content of palmitic acid with the increase of the amount of vermicompost. There is a negative correlation between the content of palmitic acid and oleic acid. The lower content of palmitic acid makes the oil suitable for dietetic purposes, while its higher content reduces the capacity and increases the level of cholesterol in the blood. The consumption of unsaturated fatty acids leads to the formation of so-called "good" cholesterine, due to which the vegetable fats are in the base of the dietary nutrition.

The impact of compost and vermicompost on the content of stearic acid depends on the amount of imported meliorant. The stearic acid content ranges from 1.73- 1.91% in the separate variants. With the increase of the amount of meliorant, the

content of stearic acid in the oil reduces. There is a slight decrease in the importation of vermicompost (up to 1.87%) in comparison to the control (1.91%).

There is a negative correlation found between the content of saturated acids - palmitic and stearic acid in the oil, which is more pronounced than that of the oleic and linoleic acid.

There are changes in the content of arachidonic, palmitoleic and linoleic acids in the oil. For example, in the oil in the variants with the importation of vermicompost there are no linolenic (C18:3), palmitoleic (C16:1), gadoleic (C20:1) and erucic (C22:1) acids.

Oil with low content of saturated acids is very suitable for culinary purposes. The content of saturated acids in the oil of the control reaches up to 8.4%, and of unsaturated acids - up to 91.6%.

The organic meliorants used influence also the ratio of saturated - unsaturated acids. The ratio of unsaturated to saturated fatty acids (U/S) in the different variants ranges from 9.97 to 10.94 (Table II). A change in this ratio is observed for both meliorants used. Increase in the content of the saturated acids is observed in all variants, as a significant increase in relation to the control (8.4%) is observed in the variants with 20 t/decare of compost (9.1%) and 20 t/decare of vermicompost (8.9%).

According to [53] the increase of N in the soil leads to an increase in the percentage of unsaturated fatty acids and decrease in the percentage of saturated fatty acids in linseed oil. Similar results were obtained by [54] who reported that the addition of nitrogen fertilizers affect the fatty-acid composition of the sunflower oil.

The impact of vermicompost on the content of saturated fatty acids is not unidirectional. For example, the addition of vermicompost leads to an increase of the content of palmitic acid and simultaneously leads to a lower content of other saturated fatty acids (stearic and arachidic). Similar are the results obtained in terms of unsaturated acids. The importation of vermicompost leads to reduction of the content of oleic, linoleic, arachidonic, gadoleic and erucic acid and an increase of the content of linoleic acid in comparison with the control.

IV. CONCLUSION

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

1. Addition of compost and vermicompost has a significant effect on reducing the heavy metal content in the seeds of safflower. The introduction of 40 t/daa of compost and 40 t/daa of vermicompost leads to the reduction of Cd to levels of 2.9 mg/kg to 0.5 mg/kg, which is within the maximum limits for forage.
2. The introduction of organic additives in the soil affects the quality of the oil. Introducing vermicompost and compost leads to an increase in the content of palmitic acid and linoleic acid, and a decrease in the stearic and oleic acids compared with the control. A significant increase in the quantity of saturated acids was observed in the variants with 20 t/daa of compost and 20 t/daa of vermicompost (9.1 and 8.9% relative to the control).

3. Safflower is a plant which is tolerant to heavy metals and can be successfully used in the phytoremediation of heavy metal contaminated soils. The processing of seeds to oil and using the obtained oil for nutritional purposes will greatly reduce the cost of phytoremediation.

ACKNOWLEDGMENT

The authors acknowledge the support of grants from Bulgarian Ministry of Education (Project DFNI T-02/4).

REFERENCES

- [1] A. P. G. C. Marques, R.S. Oliveira, A. O. S. S. Rangel, and P. M. L. Castro, "Application of manure and compost to contaminated soils and its effect on zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi", *Environmental Pollution*, vol.151, pp.608–620, 2008.
- [2] N. Haque, J. R. Peralta-Videa, G. L. Jones, T. E. Gill, and J. L. Gardea-Torresdey, "Screening the phytoremediation potential of desert broom (*Baccharis sarothroides* Gray) growing on mine tailings in Arizona, USA", *Environmental Pollution*, vol.153, pp.362–368, 2008.
- [3] I. D. Pulford and C. Watson, "Phytoremediation of heavy metal-contaminated land by trees--a review", *Environment International*, vol.29, pp.528–540, 2003.
- [4] R. L. Chaney, M. Malik, Y. M. Li, S. L. Brown, E. P. Brewer, and J. S. Angle, "Phytoremediation of soils metals", *Current Opinion in Biotechnology*, 8, pp.279–284, 1997.
- [5] J. Ciura, M. Poniedzialek, A. Sekara and E. Jedrszezyk, "The Possibility of Using Crops as Metal Phytoremediants", *Polish Journal of Environmental Studies*, vol.14, pp.17-20, 2005.
- [6] M. I. Lone, H. Zhen-Li, P. J. Stoffella, and Y. Xiao, "Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives", *Journal of Zhejiang University Sci.*, B. 9, pp. 210-220, 2008.
- [7] X. E. Yang, H. Y. Peng, and L. Y. Jiang, "Phytoremediation of Copper from contaminated soil by *Elsholtzia splendens* as affected by EDTA, citric acid, and compost", *International Journal of Phytoremediation*, vol.7, pp. 69-83, 2005.
- [8] M. Komarek, P. Tlustos, J. Szakova, W. Richner, M. Brodbeck, and M. Sennhauser, "The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils", *Chemosphere*, vol.67, pp.640–651, 2007.
- [9] A. Fässler, B. H. Robinson, S. K. Gupta, and R. Schulin, "Phytomanagement of metal-contaminated agricultural land using sunflower, maize and tobacco", *Nutrient Cycling in Agroecosystems*, vol.87, pp. 339-352, 2010.
- [10] S. Kirkova, Y. Dyulgierski, and T. Milanova, "Properties of new varieties and lines Burley tobacco investigation on consumables", *Bulgarian Journal of Agricultural Science*, vol.20, pp. 643-646, 2014.
- [11] H. Bozukov, S. Kirkova, and P. Zapryanova, "Study of technologically unusable tobacco waste and practical solutions for its recovery", *Tymyn/Tobacco*, vol.62, pp.109-114, 2014.
- [12] Y. Dyulgierski, S. Kirkova, and T.Milanova, "Comparative analysis of Expert evaluation and Chemical composition of Varieties and lines Burley Tobacco", *Agricultural Science*, vol.46, pp.47-53, 2013.
- [13] S. Pandeliev, L. Angelov, B. Stalev, and M. Papanikolau, "Szanse ekologicznej uprawy winorosli w południowych winiarskich regionach bulgarii", in *2010 Proc. IV Ogólnopolska Konferencja Winiarska. Nowosci w uprawie winorosli I produkcji win*, Sulechów, pp. 99-109.
- [14] V. Heuzé, G. Tran, P. Chapoutot, D. Bastianelli, F. Lebas, and D. Renaudeau, "*Safflower (Carthamus tinctorius) seeds and oil meal*". Feedipedia.org. A programme by INRA, CIRAD, AFZ and FAO, 2012.
- [15] A. B. Rahamatalla, E. E. Babiker, A. G. Krishna, and A. H. El Tinay, "Changes in chemical composition, minerals and amino acids during seed growth and development of four safflower cultivars", *Plant Foods for Human Nutrition*, vol.52, pp. 161–170, 1988.
- [16] A. B. Rahamatalla, E. E. Babiker, A. G. Krishna, and A. H. El Tinay, "Changes in fatty acids composition during seed growth and physicochemical characteristics of oil extracted from four safflower cultivars", *Plant Foods for Human Nutrition*, vol.56, pp. 385–395, 2001.
- [17] GRDC, "*Raising the bar with better safflower agronomy*", GRDC - Grain research and development corporation, 2010, pp.39.
- [18] V. Angelova, R. Ivanova, J. Todorov, K. Ivanov, M. Perifanova-Nemska, G. Uzunova, and V. Ivanova, "The effect of organic amendments on uptake on heavy metals in safflower (*Carthamus tinctorius* L.)", in *Proceeding 6th Symposium on recycling technologies and sustainable development*, September 18 - 21, Soko Banja, Srbija, pp.331-338, 2011.
- [19] R. Clemente, D.J. Walker, and M.P. Bernal, "Uptake of heavy metals and As by *Brassica juncea* grown in a contamination soil in Arnalcollar (Spain): The effect of soil amendments", *Environmental Pollution*, vol.136, pp.46 – 58, 2005.
- [20] V. Angelova, M. Petkova, K. Ivanov, and P. Zapryanova, "Effects of Organic Soil Amendments on Soil Physical and Chemical Properties", in *Proceeding 6th Symposium on recycling technologies and sustainable development*, September 18 – 21, Soko Banja, Srbija, pp.315-322, 2011.
- [21] D. J. Walker, R. Clemente, A. Roig, and M. P. Bernal, "The effect of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils", *Environmental Pollution*, vol.22, pp.303 – 312, 2003.
- [22] D. J. Walker, R. Clemente, and M. P. Bernal, "Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by pyritic mine waste". *Chemosphere*, vol.57, 215- 224, 2004.
- [23] Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Kjeldahl method, BS EN ISO 5983-1,2005.
- [24] Animal and vegetable fat and oils – Preparation of methyl esters of fatty acids. ISO 5509, 2000.
- [25] Animal and vegetable fat and oils – Determination of methyl esters of fatty acids (Gas chromatographic method). ISO 5508, 2000.
- [26] G. Barancikova, and J. Makovnikova, "The influence of humic acid quality on sorption and mobility of heavy metals", *Plant Soil Environ.*, vol.49, pp.565-571, 2003.
- [27] M. M. Kononova, *Soil Organic Matter*. Pergamon Press, Oxford. 1966, 2nd edition, 1966.
- [28] H. J. Hapke, "Metal accumulation in food chain and Load of feed and food", in *Metals and their compounds in the environment. Occurrence, analysis, and biological relevance*, E. Merian, Ed. New York: Weinheim, 1991, pp. 469-479.
- [29] R. L. Chaney, "Toxic element accumulation in soils and crops: protecting soil fertility and agricultural food-chains", in: *Inorganic contaminants in the Vadose Zone*, B. Bar-Yosef, NJ Barrow, J. Goldshmid J, Eds. Berlin: Springer-Verlag, 1989, pp.140 –158.
- [30] M. Penumetcha, N. Khan, and S. Parthasarathy, "Dietary oxidized fatty acids: An atherogenic rise", *J. Lipid Res.*, vol.41, pp. 1473-1480, 2000.
- [31] L. Velasco, and J. M. Fernandez-Martinez, "Breeding for oil quality in safflower", in *2001 Proceedings of the 5th International Safflower Conference*. Williston, North Dakota and Sidney, Montana, USA, 133-137.
- [32] R. P. Mensink, E. H. M. Temme, and G. Hornstra, "Dietary saturated and trans fatty acids and lipoprotein metabolism", *Ann. Med.*, vol.26, pp.461—464, 1994.
- [33] M. R. Sabzalian, G. Saeidi, and A. Mirloh, "Oil content and fatty acid composition in seeds of three safflower species", *J Am Oil Chem Soc.*, vol.85,717–721, 2008.
- [34] E. Petcu, A. Arsintescu, and D. Stanciu, "The effect of drought stress on fatty acid composition in some Romanian sunflower hybrids", *Romanian Agricultural Research*, vol.15, pp. 39-43, 2001.
- [35] E.S. Bassil, and S.R. Kaffka, "Response of safflower (*Carthamus tinctorius* L.) to saline soils and irrigation: I.Consumptive water use", *Agric. Water Manag.*, vol.54, pp.67- 80, 2002.
- [36] Z. Zhang, and Y. Chen, "Studies on adaptability of safflower germplasms in Xinjiang, China", in *VIth International Safflower Conference*, Istanbul 6-10 June 2005. pp.132-139.
- [37] S. D. Koutroubas, and D. K. Papadoska, "Adaptation, grain yield and oil content of safflower in Greece", in *VIth International Safflower Conference*, Istanbul 6-10 June 2005, pp. 161-167.
- [38] P. B. Gawand, S. I. Tambe, and B. N. Reddy, "Evaluation of productivity of safflower cultivars under moisture and nutrient management in rainfed vertisols", in *VIth International Safflower Conference*, Istanbul 6-10 June 2005, pp.205-209.
- [39] B. Arslan, and M. Küçük, "Oil content and fatty acid composition of some safflower cultivars in Van (Turkey)", in *VIth International Safflower Conference*, Istanbul 6-10 June 2005. pp. 167-175.
- [40] V. Kumar, "Nitrogen economy in Indian mustard through use of *Azotobacter chroococcum*", *Crop Research*, vol.8, pp. 449-452, 1994.

- [41] S. C. Wu, Z. H. Cao, Z. C. Li, and K. C. Cheung, "Effect of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial", *Geoderma*, vol.125, pp.155-166, 2005.
- [42] J. D. Scheiner, F.H. Gutiérrez-Boem, and R.S. Lavado, "Sunflower nitrogen requirement and ¹⁵N fertilizer recovery in Western Pampas, Argentina", *Eur. J. Agron.*, vol.17, pp.73-79, 2002.
- [43] D. T. Canvin, The effect of temperature on the oil content and fatty acid composition of the oils from several oil seed crops. *Canadian J. Bot.*, vol.43, pp. 63-69, 1965.
- [44] G. Nagaraj, "Safflower seed composition and oil quality - A review", in *Proc. 3rd International Safflower Conference*, 14-18 June 1993, Beijing, China.
- [45] M. A. Munir, M.A. Malik and M.F. Saleem, "Impact of integration of crop manuring and nitrogen application on growth, yield and quality of spring planted sunflower (*Helianthus annuus* L.)", *Pak. J. Bot.*, vol.39, pp. 441-449, 2007.
- [46] E. Petcu, E., A. Arsintescu, and D. Stanciu, "The effect of hydric stress on some characteristics of sunflower plants", *Romanian Agricultural Research*, vol.16, pp. 15-22, 2001.
- [47] P. Akbari, A. Ghalavand, A. M. Modarres Sanavy, and M. Agha Alikhani, "The effect of biofertilizers, nitrogen fertilizer and farmyard manure on grain yield and seed quality of sunflower (*Helianthus annuus* L.)", *Journal of Agricultural Technology*, vol.7(1), pp.173-184, 2011.
- [48] G. I. Seiler, "Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower", *Industrial Crops and Products*, vol.25, pp.95-100, 2007.
- [49] G. I. Seiler, "Analysis of the relationships of environmental factors with seed oil and fatty acid concentrations of wild annual sunflower", *Field Crops Research*, vol.15, 57-72, 1986.
- [50] U. Gecgel, M. Demirci, E. Esendal, and M. Tasan, "Fatty acid composition of the oil from developing seeds of different varieties of safflower (*Carthamus tinctorius* L.)", *J Am Oil Chem Soc.*, vol.84, pp.47-54, 2007.
- [51] Z. Zongwen, and C. Yuehua, "Studies on the ecological adaptability of safflower germplasms", in *Proceedings VIth International Safflowers Conference*, 6-10 June 1997, Istanbul, Turkey.
- [52] R. K. Downey, and G. F. W. Rakow, "Rapeseed and mustard", in *Principles of Cultivar Development*, W. R. Fehr, Ed. New York Macmillan Publishing Co., 1987, pp. 437-886.
- [53] N. F. Kheir, E. Z. Harb, H. A. Moursi, and S. H. El-Gayar, "Effect of Salinity and Fertilization on Flax Plants (*Linum usitatissimum* L.). II. Chemical Composition", *Bull. Faculty Agriculture (Univ. Cairo)*, vol. 42, pp. 57-70, 1991.
- [54] B. T. Steer, and G. I. Seiler, "Changes in fatty acid composition of sunflower (*Helianthus annuus* L.) seeds in response to time of nitrogen application, supply rates and defoliation", *Journal of the Science of Food and Agriculture*, vol.51, pp. 11-26, 1990.