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Full Length Research Paper

Monitoring of heavy metals and selected micronutrients in hempseeds from North-western Turkey

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Heavy metal contamination of agricultural soils and subsequent accumulation of these metals in plants is a growing interest. In this work heavy metal and selected micronutrient content (Cd, B, Al, Co, Cu, Mo, Ni, Zn, Fe, Mn, Pb and Cr) in twenty -one different hempseed samples collected from North - western Turkey was monitored. The quantitative measurements were carried out with inductively - coupled plasma (ICP-OES). The content of Pb and Cr was below the detectable levels in all the seed samples. Cadmium content of the hempseeds was found within the range of 5 - 23 μgkg^{-1}. Of the monitored trace elements, the highest concentration found was of iron (98 -121 $mgkg^{-1}$) followed by manganese (70 - 102 $mgkg^{-1}$) and zinc (46 - 72 $mgkg^{-1}$). On the other hand, the lowest concentration found was of cobalt (0.06 - 0.17 $mgkg^{-1}$) followed by molybdenum (0.25 - 0.62 $mgkg^{-1}$) and nickel (0.55 - 1.66 $mgkg^{-1}$). The results of the present study reveal that heavy metal contents of the hempseeds from North-western Turkey are well below the critical levels established for safe human consumption. On the other hand, selected micronutrient profile obtained in this study also shows that hempseeds appears be a potentially valuable food source for human nutrition.

Key words: Hemp (*Cannabis sativa* L.), trace elements, nutrition, food source, toxicity.

INTRODUCTION

In the last few decades, growing interests in environmental concerns in connection with human and animal health have prompted a renewed focus on trace elements. Some of the trace elements including iron, manganese, zinc and copper are essential micronutrients with a variety of biochemical functions in all living organisms. However, the benefits of these micronutrients may be completely reversed if present at high concentrations (Bennett, 1993; Marschner, 1995). Some heavy metals particularly cadmium and lead, not essential elements for human nutrition, on the other hand, have been considered serious soil and environment pollutant due to their potential toxicity at low concentrations (Das et al., 1997; Caldas and Machado, 2004).

Heavy metals naturally present at low concentrations in agricultural soils. Due to their widespread occurrence, cumulative behavior and toxicity, however, they are of considerable importance with a potential hazardous effect on both crop plants and human health (Nriagu, 1990; Shi and Cai, 2009). A number of factors contribute to heavy metal contamination of agricultural soils including industrial and traffic emission, atmospheric deposition from town wastes, using metal-containing agricultural expedients and metal production (Alloway and Jackson, 1991; Latinca et al., 2004). Contamination of agricultural soils with heavy metals and a consequent uptake and accumulation of these metals by plants, particularly in the edible parts, has long been a critical issue. Heavy metal ions in contaminated soils may easily enter the human food chain through crop plants,

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depending on geochemical characteristics of the soil and the ability of plants to selectively accumulate some of these elements (Peris et al., 2007).

Plant species greatly differ in their ability to uptake, accumulate and tolerate heavy metals and this variation occurs not only among plant species but also within a given species (Raskin et al., 1997; Angelova et al., 2004). The heavy metal accumulation by crop species decreases in the following order: leaf vegetables > root vegetables > grain crops (Puschenreiter et al., 2005). Such differences can occur between different parts of the crops and the edible parts are the most relevant as heavy metals can be easily transferred from them to the human food chain. Except for roots, the highest concentrations are found in leaves, whereas the lowest are typically observed in seeds (Ivanova et al., 2003). Recently, there is great interest in the identification of plant species capable of accumulating high amounts of heavy metals in their tissues, with the aim of employing them for phytoremediation of contaminated soils (Gisbert et al., 2006; Salt et al., 1998). In this context, hemp plant (*Cannabis sativa* L.) provides a good alternative for soil phytoremediation since it has a high above ground biomass with about 1 m deep roots that grows fast and easily (Cittero et al., 2003; Yanchev et al., 2000; Linger et al., 2005). Hemp, a multi-use plant, has been an important source of food, fiber, oil and medicine for thousands of years.

Hempseed, with unique nutritional properties, has commonly been claimed as one the most nutritionally complete food source (Deferne et al., 1996). No other single plant source has a full amino acid spectrum in such an easily digestible form, nor has the essential fatty acids in as perfect ratio to meet human nutritional needs and has a massive trace mineral con-tent as well (Callaway, 2004; Leizer et al., 2000). Whole hemp seed contains approximately 25 - 35% oil, 20 - 25% protein, 20 -30% carbohydrates and 10 -15% insoluble fiber, as well as a rich array of minerals, parti-cularly phosphorus, potassium, magnesium, sulfur and calcium, along with modest amounts of iron and zinc (Deferne et al., 1996; Callaway, 2004; Leizer et al., 2000; Oomah et al., 2002). Hempseed's properties have long been recognized and valued as food for both hu-mans and domesticated animals throughout Asia, India, Russia and Eastern Europe (Callaway, 2004). In China, roasted hempseed is still sold as snacks by street venders. Similarly, roasted hempseed, Turkish name is çedene, mixed with roasted bread wheat (Turkish name is kavurga) has long been traditionally used as snacks in country sides of Turkey. There are a number of studies focusing on heavy metal and micronutrient content of hempseeds from different countries. However, no scien-tific paper reporting the heavy metal and micronutrient concentrations of hempseeds from Turkey has been published. We thus monitored twenty one hempseed samples collected in North-western region of Turkey, the most important region for hemp seed production, for their heavy metal and some selected micronutrient content.

MATERIALS AND METHODS

Seed accessions of hemp were obtained in October 2007 from seed wholesalers and local spice shops in North-western Turkey (Taşköprü, Vezirköprü, Gümüşhacıköy, Ordu, Çorum, Samsun and Ereğli provinces or towns), the most important region for hemp seed production. Three different seed samples of 300 g were taken in each sampling site, giving a total of 21 seed accessions. For chemical analysis, 0.2 g of each ground sample was put into a burning cup with 5mL HNO_3 65% (Merck, Darmastadt, Germany), and 2 mL H_2O_2 30%, (Merck).

The samples were incinerated in an HP - 500 CEM MARS 5 microwave (Mathews, NC, USA) at 200°C and cooled at room temperature for 45 min. The extracts were passed through a Whatman 42 filter paper, and the filtrates were collected by high deionised water in 20mL polyethylene bottles and kept at 4°C, in the laboratory, for inductively coupled plasma – atomic emission spectrometry (ICP–OES) analysis. Each sample was analyzed in triplicate. Merck standards (R1 and R2 groups) were used as analytical reagent grade chemicals. Standard solutions of Fe, Mn, Zn, Cu, B, Mo, Ni, Co, Pb, Cr, Al and Cd were prepared in 1% HNO3 immediately before the analysis by serial dilution of 1000 mg L^{-1} stock solution stored in polyethylene bottles. Peach leaves (Standard Reference Material, 1547) and corn bran (Standard Reference Material, 8433) was used as reference materials (N.I.S.T, 2004).

Scanning ICP - OES (Varian Vista-Pro, Australia) with high resolution nitrogen purged with 1 m monochromator was used. Fe, Mn, Zn, Cu, B, Mo, Ni, Co, Pb, Cr, Al and Cd contents were determined using ICP-OES. In addition, to determine cadmium concentration in the extracts, inductively coupled argon plasma - optical emission spectrometer (ICP - OES; U - 5000AT+ Ultrasonic Nebulizer; Cetac Technologies, Omaha, NE, USA) (214.438 nm/0.1 $\mu g kg^{-1}$) was also used. Analytical recovery of the method has been checked by a parallel analysis of the two certified reference materials.

RESULTS AND DISCUSSION

In this study twenty-one different hempseed samples collected in North-western Turkey, the most important region for hempseed production, were subjected to chemical analysis for their trace elements and heavy metal content. The mean values of selected trace elements and heavy metals in hempseeds obtained from each sampling site are presented in Tables 1 and 2, respectively. The values, based on plant's dry weight, are the means of three replicates and given as mean ± SD. The correlation coefficients of the calibration curves were generally within the range of 0.996 - 0.999 and acceptable recoveries (>95%) were obtained for the analysis.

The concentration of lead and chrome in the seed samples monitored was below the limit of quantification. Of the monitored trace elements, the highest concentration found was that of iron followed by manganese and zinc. On the other hand, the lowest concentration found was that of cobalt followed by molybdenum and nickel. The values for boron and copper content in

Table 1. Concentration of Al, Ni, Co and Cd in hempseeds from North-western Turkey.

	Al	Ni	Co	Cd
		µg kg^{-1}		
Ereğli 1	24 ± 3.67	0.92 ± 0.25	0.06 ± 0.02	12 ± 0.51
Ereğli 2	20 ± 0.91	1.03 ± 0.19	0.07 ± 0.02	13 ± 3.99
Ereğli 3	21 ± 0.57	1.16 ± 0.26	0.06 ± 0.02	14 ± 5.05
Gümüşhacıköy 1	6 ± 0.20	0.65 ± 0.10	0.12 ± 0.03	15 ± 4.10
Gümüşhacıköy 2	5 ± 0.95	0.66 ± 0.10	0.10 ± 0.03	8 ± 0.22
Gümüşhacıköy 3	5 ± 1.14	0.55 ± 0.08	0.09 ± 0.03	5 ±1.80
Samsun 1	17 ± 2.83	1.06 ± 0.00	0.07 ± 0.01	17 ± 2.22
Samsun 2	14 ± 2.72	1.13 ± 0.20	0.06 ± 0.01	22 ± 4.68
Samsun 3	17 ± 2.85	0.72 ± 0.05	0.06 ± 0.02	20 ± 2.33
Vezirköprü 1	13 ± 2.13	1.66 ± 0.91	0.06 ± 0.01	7 ±1.79
Vezirköprü 2	13 ± 1.24	0.97 ± 0.08	0.17 ± 0.05	19 ± 4.36
Vezirköprü 3	10 ± 0.83	1.58 ± 0.77	0.12 ± 0.02	18 ± 2.50
Ordu 1	16 ± 3.73	1.08 ± 0.00	0.06 ± 0.00	13 ± 2.62
Ordu 2	11 ± 0.35	0.85 ± 0.14	0.07 ± 0.02	19 ± 0.56
Ordu 3	12 ± 0.11	0.72 ± 0.18	0.05 ± 0.02	6 ± 0.30
Çorum 1	18 ± 1.19	0.98 ± 0.34	0.14 ± 0.08	15 ± 1.85
Çorum 2	13 ± 1.65	1.27 ± 0.20	0.08 ± 0.00	15 ± 1.26
Çorum 3	12 ± 2.67	0.36 ± 0.04	0.07 ± 0.02	20 ± 1.64
Taşköprü 1	11 ± 1.43	1.24 ± 0.48	0.07 ± 0.02	23 ± 4.38
Taşköprü 2	13 ± 1.09	1.36 ± 0.22	0.13 ± 0.00	13 ± 1.60
Taşköprü 3	14 ± 4.15	1.33 ± 0.14	0.11 ± 0.04	13 ± 1.00

* Pb, Cr are below the detection limit.

monitored seed samples showed the lowest variation. Cadmium content in hempseed samples was found within the range of 5 -23 µg kg^{-1} and more than half of the samples had Cd concentration between 12 and 19 µg kg^{-1}. Cadmium concentration of the seed samples collected from Gümüşhacıköy was much lower than those of the other sampling sites. Cadmium, a series soil and environment pollutant, has attracted the most attention due to its potential toxicity at low concentrations. Cadmium is not an essential element for human nutrition; on the contrary, higher Cd intake may result in serious illnesses lungs, livers and kidneys (Marschner, 1995). According to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), Cd is tolerable at 100 µg kg^{-1} in cereals and food legumes, and at 100 and 300 µg kg^{-1} in medicinal plants (WHO, 1999; FAO/WHO, 1993). The highest concentration of Cd recorded in our work, 23 µg kg^{-1}, is much lower than the upper limit of 300 µg kg^{-1}, recommended for safe human consumption. The ranges of Cd content obtained in this study were also much lower than that reported in the previous studies. There is no literature about cadmium contents in hempseeds from Turkey. However, when compared with the previous studies in medicinal plants from Turkey, the cadmium levels we measured were much lower (Ozkutlu et al., 2006; Divrikli et al., 2006; Ozkutlu, 2008; Sekeroglu et al., 2008). Cadmium contents of hemp-seeds from Nigeria and Germany were reported as 2.40 and 1.1 mg kg^{-1}, respectively (Eboh and Thomas, 2005; Linger et al., 2002). A study from Bulgaria, conducted at two different distances (0.5 and 15 km) to an area polluted by heavy metals, revealed that cadmium content in hempseed decreased (from 1.0 to 0.34 mg kg^{-1}) as the distance from the source of pollution increased (Angelova et al., 2004). Boron content in the seed samples collected was quite similar to each other, within the range of 11-14 mg kg^{-1}. All twenty-one seed samples analyzed contained detectable levels of Al, varying between 5 and 24 mg kg^{-1}. Cobalt and molybdenum levels in all the samples were in the range of 0.05 - 0.17 and 0.26 - 0.62 mg kg^{-1}, respectively. In trace amounts, nickel may be beneficial in activating some enzyme systems, but its toxicity at higher levels is more prominent (Divrikli et al., 2006). Nickel toxicity in humans, however, is not a very common occurrence since the absorption level of nickel is very low (Onianwa et al., 2000). Nickel contents in the seed samples were in the range of 0.36-1.66 mg kg^{-1}. In the previous studies, the concentrations of nickel in hempseeds were reported as 6.8 by Eboh and Thomas, (2005) and 2.9 mg kg^{-1} by Linger at al. (2002).

The micronutrients Cu, Fe, Mn, and Zn are not only

Table 2. Concentration of Cu, Zn, Fe, Mn, B and Mo in hempseeds from North-western Turkey.

	Cu	Zn	Fe	Mn	B	Mo
			µg kg^{-1}			
Ereğli 1	11 ± 0.86	55 ± 3.88	116 ± 5.06	81 ± 16.63	14 ± 0.74	0.39 ± 0.01
Ereğli 2	10 ±1.22	58 ± 6.76	121 ± 8.10	79 ± 7.83	13 ± 0.89	0.46 ± 0.11
Ereğli 3	10 ±1.91	55 ± 5.07	110 ± 8.21	70 ± 0.30	13 ± 0.80	0.49 ± 0.17
Gümüşhacıköy 1	9 ± 0.69	72 ± 4.11	101 ± 3.77	92 ± 0.13	11 ± 0.30	0.29 ± 0.02
Gümüşhacıköy 2	9 ±0.20	69 ± 2.92	98 ± 15.05	92 ± 4.61	12 ± 1.00	0.27 ± 0.04
Gümüşhacıköy 3	9 ± 0.37	65 ± 6.21	102 ± 7.57	91 ± 4.34	12 ± 0.93	0.25 ± 0.06
Samsun 1	10 ± 0.87	47 ± 3.48	111 ± 8.75	83 ± 3.28	13 ± 0.39	0.59 ± 0.13
Samsun 2	10 ± 0.33	46 ± 4.27	102 ± 7.52	83 ± 3.65	12 ± 0.76	0.62 ± 0.14
Samsun 3	11 ± 0.95	47 ± 5.50	116 ± 5.82	79 ± 6.16	12 ± 0.82	0.62 ± 0.02
Vezirköprü 1	11± 1.50	58 ± 6.04	101 ± 3.10	84 ± 6.61	13 ± 1.94	0.46 ± 0.05
Vezirköprü 2	11 ± 0.84	60 ± 3.19	116 ± 12.25	90 ± 2.09	13 ± 1.16	0.41 ± 0.04
Vezirköprü 3	11 ± 1.02	60 ± 4.82	102 ± 7.71	102 ± 1.67	13 ± 1.29	0.46 ± 0.07
Ordu 1	10 ± 0.22	50 ± 2.57	110 ± 13.37	102 ± 2.97	13 ± 0.84	0.37 ± 0.05
Ordu 2	12 ± 2.73	49 ± 3.18	110 ± 0.82	98 ± 2.94	13 ± 0.80	0.51 ± 0.02
Ordu 3	11 ± 1.23	52 ± 1.70	112 ± 3.20	85 ±11.15	13 ± 0.54	0.54 ± 0.01
Çorum 1	11 ± 0.99	58 ± 1.16	121 ± 9.66	81 ± 5.28	13 ± 0.87	0.44 ± 0.01
Çorum 2	10 ± 1.25	60 ± 3.36	108 ± 8.30	78 ± 6.44	13 ± 0.41	0.40 ± 0.10
Çorum 3	10 ± 0.12	60± 3.30	106 ± 10.53	77 ± 1.74	14 ± 1.73	0.40 ± 0.02
Taşköprü 1	10 ±0.92	64 ± 2.31	108 ± 2.53	84 ± 6.25	13 ± 1.32	0.40 ± 0.07
Taşköprü 2	10 ± 1.25	59 ± 9.73	106 ± 10.36	77 ± 7.50	13 ± 1.78	0.38 ± 0.13
Taşköprü 3	11 ± 0.52	65 ± 6.09	109 ± 6.14	82 ± 15.23	13 ± 1.48	0.44 ± 0.18

photosynthesis and respiration. However, in excess amounts, these elements are toxic to plants (Bennett, 1993; Marschner, 1995). On the other hand, these trace elements are significant for human physiology when they are captured in desired levels. Copper and zinc are essential micronutrients for living organisms due to a wide range of biological functions as component of redox and enzymatic systems, the latter of which is an important enzyme co-factor for human fatty acid (FA) metabolism (Deferne et al., 1996; Mclaughlin et al., 1999). Zinc deficiency resulted from inadequate dietary intake is of growing concern in the developing world. Concentrations of copper and zinc were generally high in all hempseed samples, ranging from 9 to 12 mg kg^{-1} for copper and from 46 to 72 mg kg^{-1} for zinc. Copper and zinc contents of hempseeds from Bulgaria were reported within the range of 5.9-8.9 and 7.3-17.8 mg kg^{-1}, respectively (Angelova et al., 2004). Callaway (2004) found that copper and zinc levels in the seed of Finola hemp variety were 2 and 7 mg 100 g^{-1}, respectively. Of all the micronutrients, iron which is an important component of hemoglobin in human is required by plants in the largest amount. The iron content in the seed samples in our study was relatively high and in the range of 98 and 121 mg kg^{-1}. The concentrations of iron in hempseeds were given as 7.8 mg kg^{-1} by Eboh and Thomas (2005) and 14 mg 100 g^{-1} by Callaway (2004). Manganese is an important element activating numerous essential enzymes. Our manganese values ranging from 70 to 102 mg kg^{-1} are in agreement with those reported in the literature (Callaway, 2004; Puschenreiter et al., 2005). The recommended dietary allowances (RDA) for iron, zinc, copper and manganese are 18, 15, 2 and 5 mg d^{-1} person^{-1}, respectively (NRC, 1989). The levels of Fe, Zn, Cu and Mn in hempseed samples from North-western Turkey were lower than the recommended values given above.

Conclusion

Heavy metal and micronutrient contents of different kinds of food sources can serve a useful guidance in determining the convenience of including foods in human diet. The results of this study reveal that heavy metal concentration of hemp seeds from north -western Turkey are well below the levels established as critical for human consumption. Therefore, heavy metal content of hempseeds consumed as snacks in Turkey may not be considered a critical issue for human health. However, regular monitoring of heavy metals in hempseeds is advisable to be aware of potential risk in the future. When compared with previous studies of the micro- nutrients Cu, Fe, Zn and Mn in hempseeds from the other parts of the world, the levels we recorded for all the micronutrients mentioned were generally higher. Therefore, selected micronutrient profile obtained in this study shows that hempseeds used as snacks in Turkey appears be a potentially valuable food source for human nutrition.

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