

Evaluation of soil properties, irrigation and solid waste application levels on Cu and Zn uptake by industrial hemp

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Abstract. A three-year experiment was performed to study the alteration of copper and zinc levels in industrial hemp grown in different soils using elevated sewage slurry solid waste applications. Two soil samples, an acidic and an alkaline one, with different soil properties, such as percentage of CaCO₃ and cation exchange capacity values, were used. Three treatments of waste solid with provided elevated concentrations of Cu and Zn were combined with two irrigation levels. The application of high doses of the solid residue as well as high irrigation level lead to an increase of the mobility of metals in hemp leaves in acidic soil in contrast to alkaline. On the contrary, in alkaline soil along with a reduced irrigation level, there is a decrease in the mobility of Cu and therefore its accumulation in the roots or stems was observed. Concluding, hemp seem to be a promising plant remediator, after the application of the proper irrigation level and taking into account the physico-chemical soil properties of moderately contaminated (with copper and zinc) soils.

Key words: Heavy metals, *Cannabis sativa*, sewage sludge, phytoremediation.

INTRODUCTION

Industrial hemp (*Cannabis sativa subsp. Sativa*) is an annual herb cultivated for versatile industrial purposes such as the production of natural constructive and insulating materials from the fibres or making paints, varnishes and soaps from the oil contained in seeds. Industrial hemp seeds, high in protein and oil, are also edible and are considered a healthy food supplement (Wogiatzi et al., 2019). Industrial hemp has also outstanding features like high biomass yield in relatively short time, biomass of high calorific value and low input requirements making it suitable as an energy crop (Kolarikova et al., 2015).

During the last decades heavy metal contamination of agricultural soils is one of the most important environmental problems (Kabata-Pendias, 2011; Alloway, 2013; Murtic et al., 2019). Heavy metal present and persistence nature may have dangerous

effects on wild life and human life as well (Craven et al., 2019). On the other hand, phytoremediation technologies (such as phytostabilization, phytodegradation, phytoextraction and phytovolatilization) use plants and wild trees to ameliorate contaminated soils (Citterio et al., 2003; Murtic et al., 2019). Industrial hemp could be a potential soil phytoremediation agent, as it is a tall plant with about one-meter-deep roots that grows fast and easily in dense stands, with high biomass production potential and extracts heavy metals (Moghaddam et al., 2020).

Sewage sludge is a solid waste by-product obtained from urban and industrial wastewater treatment plants. Traditionally it is disposed on landfills either as raw material or after incineration. Its high nutrient concentration (N, P, S, Fe, Mg and Ca) however, evoked the challenge to use it as an agricultural amendment to improve soil fertility and soil structure (Breda et al., 2020). Sewage sludge however, contains also potentially toxic compounds, such as heavy metals and persistent organic pollutants. Long term use as a soil amendment may impose serious risks of heavy metal soil contamination (Singh & Agrawal, 2008; Praspaliauskasa et al., 2020). The application of sewage sludge, produced by the municipality of Volos and Almyros, in industrial hemp plants cultivation, may be a good and cheap alternative to the fertilizer's application improving soil quality (Lazdina et al., 2011; Zielonka et al., 2020).

Moreover, there is a lack of knowledge regarding industrial hemp mechanisms for accumulation and tolerance to high or moderate contaminant concentrations in the soil (Safari Singani & Ahmadi, 2012; Zerihun et al., 2015). Furthermore, it is well known that industrial hemp is a crop with high water demands and irrigation is essential in low rainfall environments such as southern Europe (Amaducci et al., 2015). Irrigation regimes play an important role in heavy metals mobility into the soil enhancing their leaching to groundwaters (Haykiri-Acma et al., 2011; Ahmad et al., 2018; Azouzi et al., 2019). Soil moisture levels along with pH and organic matter values are crucial in accumulation of heavy metals (Stafford et al., 2018). Enhanced heavy metal absorption was noticed under alternate irrigation (wetting and drying) by Song et al., 2021, while metal absorption by plants is increased under ample soil water regimes (Angle et al., 2003; Marchiol et al., 2007; Wogiatzi et al., 2019).

The aim of the present study was to evaluate the ability of industrial hemp to extract copper and zinc from different soil types, dressed with different doses of municipality sewage sludge and therefore, achieving different concentrations of the heavy metals. The effect of the soil water regime was also considered by introducing two alternative irrigation treatments.

MATERIALS AND METHODS

The experiment was conducted at the farm of University of Thessaly from 2018–2020. The farm is located near the Athens-Thessaloniki National Road, in the area of Velesino, Magnesia (39° 23' 44" N, 22° 45' 26" E), at an altitude of 70 m above sea level and covers an area of 15 hectares. First seeding took place in April 2018 applying industrial hemp (*Cannabis sativa subsp. sativa*) seed cultivar 'Futura 75'. Due to the non-uniform emergence in few experimental plots and in order to achieve the same population in all plots, the plants were diluted to a population of approximately 90 plants m⁻². The mature plants were harvested about 16 weeks later, with an average stem height of 125 cm. Soil 1 (S1) is the native of the farm, while for soil 2 (S2) a mixture of farm soil with soil

collected from cultivated fields at the immediate vicinity of the industrial area of Volos was used in 1:3 ratios. The waste solid residue is part of the sludge obtained by the local Biological Treatment Industry (Municipality of Volos and Almyros). The plant operates around 30,000 m³ per day of raw sewage which undergoes primary physical, biological and chemical treatments including screening, thickening, dewatering, anaerobic and aerobic digestion alkaline stabilization and composting that led to a stabilized solid waste with around 90% of solids (Gudulas et al., 2007). The solid waste analyzed for its chemical, physical properties and the results are presented in Table 1. The solid waste was incorporated into the experimental plots by means of a rotary cultivator.

A uniform sprinkler irrigation was applied to all the plots immediately after sowing to facilitate crop emergence. Thereafter, a drip irrigation system was established. Drip pipes with drips at 40 cm and a capacity of 2 L h⁻¹ were placed every second crop row. The pipe network was divided into two subnetworks to fulfil the needs of the experiment. The evapotranspiration (ET_f) was measured using Thornthwaite method, while field capacity, permanent wilting point, available soil moisture and critical soil moisture was calculated (Zhang et al., 2020). The above data were used to calculate the irrigation dose, while the field soil moisture was measured using TDR. When soil moisture reached critical soil moisture, an irrigation event was planned. Mean ET_f value in the farm was 435 mm. Six to eight irrigation events were performed each year, depending on the weather conditions. Totally, for the 100% ET_f treatment, 320 mm of irrigation water were applied in 2018, 450 mm in 2019 and 450 mm in 2020, while total seasonal precipitation was 262.2 mm, 108.2 mm and 108 mm respectively. Half of these doses were applied on the 50% ET_f treatment.

Three treatments of solid waste disposal (W₁ = 3.5 kg m⁻², W₂ = 7 kg m⁻² and W₃ = 10 kg m⁻² incorporated 21.8, 43.8 and 62.5 kg per plot in each treatment, respectively) providing elevated concentrations of Cu and Zn were combined with two irrigation levels (I₁ = 50% ET_f and I₂ = 100% ET_f) over two soil types (S₁ = alkaline, S₂ = acidic). Two more treatments, one for each soil type, received 100% ET_f irrigation but no solid waste disposal and used as a control (C). As a result, a total of 3×2×2+2 = 14 treatments were founded and arranged in a complete randomized block design with three side by side replications. The plot size was 6.25 m² (2.5×52.5 m wide; 10 rows per plot and 56 plants per row) with a total area of the experiment including corridors and margins, of 0.04 ha. All the treatments were conducted in three replicates.

During harvest period (16 weeks after emergence), 20 plants were uprooted from the inner plot in case to avoid the border effect. Thereafter to separate the root system from the soil, the root was placed in aqueous sodium polymethosphate solution for five days for structure dissolving. Then the soil was rinsed with plenty of water and finally only the root system remained. After harvest, a partitioning of the plants took place and hemp parts were placed in a dry chamber at 50 °C until a constant weight was reached. Soil samples were collected, dried and both soil and hemp parts were analysed for Cu and Zn. Soil samples were analysed (Page et al., 1982) for: pH and electrical conductivity (EC) (1:2.5 H₂O), organic matter (Walkley and Black method), and cation exchange capacity (CEC, 1M CH₃COONH₄, pH7). The CaCO₃ (%) was determined using the Bernard method. Soil and hemp samples were mineralised with 3 mL of concentrated HNO₃, 3 mL of HF and 1 mL of HCl at 750 Watt, 6 MPa, in a microwave digestion system (JAOAC 1984). The analysis of the extractants prepared from the soil, roots, stems and leaves samples were performed using an ICP-OES (Inductively Coupled

Plasma - Optical Emission Spectrometry) (Thermo Scientific). Determinations in each of the analysed samples were carried out in three replications. For the data acquisition of the samples, a quantitative analysis mode was used. Certified material to test the analytical method (NIST 1573a, tomato leaves) was used.

A One-way Anova was applied to analyze the data using the SPSS-v26 suite. The LSD and Tuckey's test ($p < 0.05$) were applied for post-hoc analysis and mean separation. Graphs were built on Microsoft Excel.

RESULTS AND DISCUSSION

Table 1 shows the physico-chemical composition of the two soil samples as well as the waste solid used in the study. The soil sample of the university farm characterized as clayloam and has a pH value 7.6 (Soil 1), while soil 2 has a pH value of 5.9. The content of organic matter is rather low in both soils, a characteristic property of Greek soils that have undergone long-term tillage. Soil 1 has a low CaCO_3 content, while in soil 2 there was no CaCO_3 detected. The Cation Exchange Capacity (CEC) and clay content is higher in the 1st soil sample.

Table 1. Chemical, physical properties and Cu and Zn concentrations of control soil samples and waste solid

Treatments	Soil 1	Soil 2	Waste solid
pH (1:2.5)	7.6 ± 0.2	5.9 ± 0.3	8.4 ± 0.1
Organic matter (OM) (%)	1.6 ± 0.1	1.7 ± 0.2	24 ± 1.1
Bulk specific gravity (g cm^{-3})	1.3 ± 0.3	1.2 ± 0.4	2.3 ± 0.2
CaCO_3 (%)	4.8 ± 0.4	ND	9.3 ± 0.3
CEC ($\text{cmol}_c\text{kg}^{-1}$ soil)	29 ± 0.5	21 ± 0.2	52 ± 0.5
Total Cu (mg kg^{-1} dry soil-dry matter*)	4.3 ± 0.5	3.5 ± 0.3	67.4 ± 1.2*
Total Zn (mg kg^{-1} dry soil-dry matter*)	5.2 ± 0.7	4.5 ± 0.4	75.8 ± 2.1*

($n = 9$; mean of the three years of the study ± RSD is reported; ND: not detected).

The analysis of variance showed no significant differences among the years so the average values are presented. Waste solid application dose has a significant ($p < 0.05$) effect on the heavy metals concentrations in the soil, as metal concentrations in the soil increased along with waste solid application dose. The highest increase in heavy metals concentrations after waste solid application was for Cu, while the effect on the Zn concentrations was less pronounced (Table 2).

Table 2. Cu and Zn composition of soils after treatments with waste solid (mean of the three years of the study). (Waste solid doses: 1–3.5, 2–7, 3–10 kg m^{-2})

Treatments		Cu	Zn
		mg kg^{-1} dry soil	
W1: 3.5 kg m^{-2}	Soil 1	8.6 ± 0.9	10.2 ± 1.1
W2: 7 kg m^{-2}	Soil 1	19.9 ± 1.3	22.4 ± 1.4
W3:10 kg m^{-2}	Soil 1	31.9 ± 2.4	35.2 ± 2.1
W1: 3.5 kg m^{-2}	Soil 2	8.3 ± 1.2	9.1 ± 2.1
W2: 7 kg m^{-2}	Soil 2	17.3 ± 0.9	19.3 ± 0.8
W3:10 kg m^{-2}	Soil 2	29.2 ± 1.4	32.4 ± 1.3
EU limits (86/278/EEC Directive)		70–140	300
(Council European Communities, 1986)			

Copper and Zinc concentrations range at trace levels lower than the Maximum Allowable Concentrations defined in the European Council Directive 86/278/EEC. Cumulative Cu and Zn uptake by hemp plants during the growth period (four months) is presented in Figs 1 and 2. The increase of Cu reaches at 746.4% while Zn increase is up to 613.7% in regarding control soil 2. Cu and Zn in hemp parts were higher than the initial concentrations (control soils), due to the higher content of these metals in waste solid applied in the soils. It is apparent that industrial hemp absorbs Cu and Zn in roots, stems and leaves as well, in different amounts, with respect to different irrigation levels and waste solid treatments (Wogiatzi et al., 2019). The largest amounts of Cu were accumulated in the roots and stems, while Zn was accumulated in all parts of the industrial hemp and is higher than Cu in all tissues (Moghaddam et al., 2020). In alkaline soil (S_1) (Fig. 1) the retention of both copper and zinc by the solid phase of soil is stronger. This is probably due to the high percentage of clay content and the high value of the Cation Exchange Capacity (CEC) as well. As a consequence, copper concentrations were higher in the roots than in stems or leaves of the plants. On the other hand, zinc seems to be more mobile as higher zinc concentrations were measured both in stems and leaves. Accumulated Zn in hemp was rather equally distributed among the roots, stems and leaves. The highest increase in Zn concentration was detected in leaves, both for $I_2W_3S_1$ (in soil 1, Fig. 1) and $I_2W_3S_2$ (in soil 2, Fig. 2) treatments, 269.6% and 277.6% respectively.

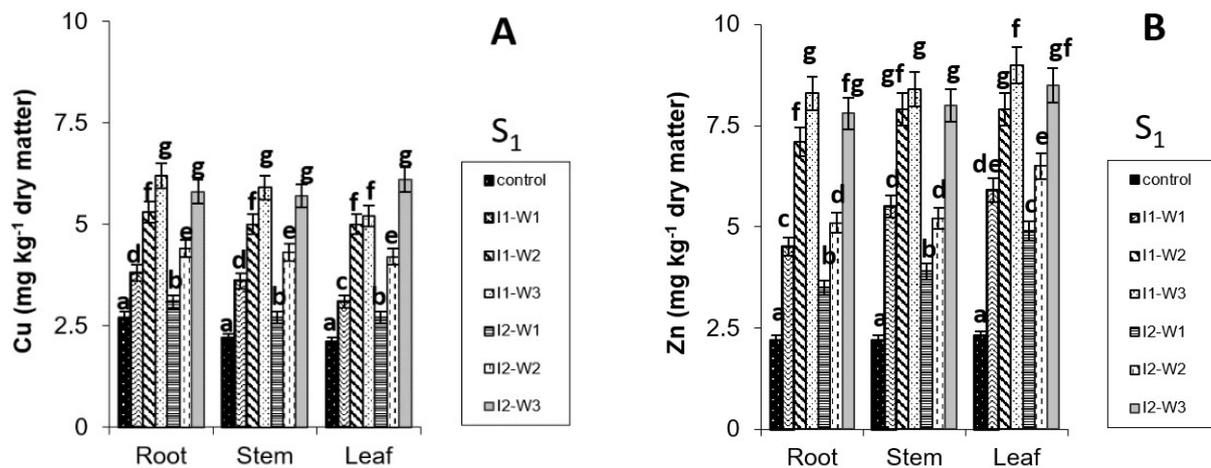


Figure 1. Mean concentrations of Cu (A) and Zn (B) in roots, stems and leaves of industrial hemp after irrigation and solid waste treatments application in alkaline soil S_1 (three years' average values) I_1 : 50% ET_f , I_2 : 100% ET_f , W_1 : solid waste 3.5 $kg\ m^{-2}$, W_2 : solid waste 7 $kg\ m^{-2}$, W_3 : solid waste 10 $kg\ m^{-2}$. Vertical lines at top of the bars denote LSD at $p < 0.05$, different letters 'a', 'b' 'c' e.t.c. denote a significant difference according to the post-hoc Tukey's test at $p < 0.05$.

In acid soil (S_2) the bioavailability of metals increases. This is shown in Fig. 2, where the concentrations of copper and zinc transferred from soil to plant are higher than in soil 1, despite the fact that the initial metal concentrations do not differ statistically significantly between soil 1 and soil 2 (Table 1 & 2). The increase in Cu concentration in industrial hemp was correlated to waste solid application and irrigation rates as well. The copper concentration gradually increased from 40.7% to 129.6% in the roots compared with the control (Fig. 1). Regardless of Cu low mobility, there was an increase in Cu concentration in the leaves in I_2W_3 treatment, which reached 190.5% up to control

soil 1. The elevated irrigation level (I_2) enhance copper solubility and translocation of copper from soil to the leaves of the plant. Irrigation regimes influences metal mobility, accelerating cations' movement to aerial tissues (Ahmad et al., 2018). The increase of Cu concentration in the stem of the plant is also quite important, regardless the fertilization intensity. Relatively high stem Cu concentrations could be explained by metals translocation via stems (Kabata-Pendias, 2011; Alloway, 2013; Murtic et al., 2019).

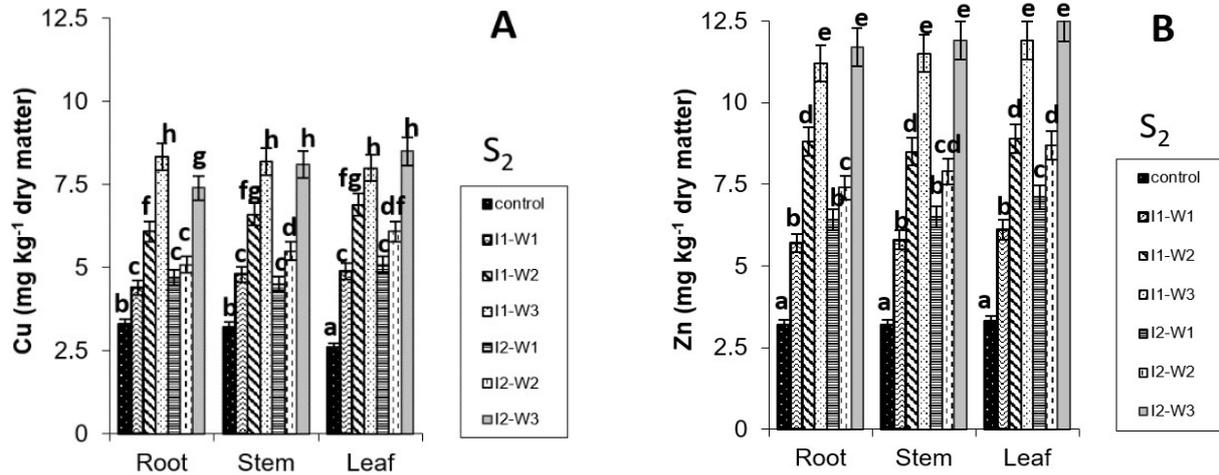


Figure 2. Mean concentrations of Cu (A) and Zn (B) in roots, stems and leaves of industrial hemp after irrigation and solid waste treatments application in acidic soil S_2 (three years' average values) I_1 : 50% ET_f , I_2 : 100% ET_f , W_1 : solid waste 3.5 kg m⁻², W_2 : solid waste 7 kg m⁻², W_3 : solid waste 10 kg m⁻². Vertical lines at top of the bars denote LSD at $p < 0.05$, different letters 'a', 'b' 'c' e.t.c. denote a significant difference according to the post-hoc Tukey's test at $p < 0.05$.

In soil 1 Cu concentration in I_1 treatment follows the order roots>stems>leaves, while in high irrigation treatment (I_2) the order changes to leaves>stem>roots. For Zn the order is leaves>stem>roots, both in acid and alkaline soil regardless the irrigation level applied. The comparison of metals accumulation from waste solids application to soils by energy plants has revealed that Zn accumulation was the highest one (Breda et al., 2020; Praspaliauskasa et al., 2020). The availability of metals in soil is influenced by soil properties, as well as water content (Azouzi et al., 2019). The chemical form of heavy metals in soil significantly affects their translocation in plant parts (Alloway, 2013; Ahmad et al., 2018). Both copper and zinc cations are soluble in soil solution and their mobility increases as the irrigation level rises. The amount of water added in combination with fertilization practices seem to be the key parameter for Zn availability and transportation from soil to plant (leaves) (Lazdina et al., 2011; Wogiatzi et al., 2019). Soil pH along with clay content are considered the most important soil factors determining the concentration of metals in the soil solution, and thus their mobility and availability to plants.

CONCLUSIONS

Concentrations of Cu and Zn in industrial hemp parts (roots, stems and leaves) are significantly affected by soil physico-chemical parameters and especially by soil pH. In the acidic soil and, after the maximum irrigation level application, Zn has sufficiently moved from the soil to the industrial hemp leaves, while when the amount of water was reduced at 50%, higher concentrations of Cu were observed in the roots and stems of the plants. In alkaline soil the mobility was lower and the movement towards the leaves was due to the increase of irrigation water level. The present 3-year study assessed that industrial hemp could be used for phytoextraction purposes of moderately contaminated soils, after controlling the amount of irrigation water added, along with the solid waste levels application.

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