Influence of zeolite and *Posidonia oceanica* (L.) in reduction of Cadmium uptake by tobacco (*Nicotiana tabacum*) plants of Central Greece

E.E. Golia[1]*, G. Fuleky[2], A. Dimirkou[1], G. Gizas[3]

[1]University of Thessaly
Department of Agriculture, Crop Production and Rural Environment
Laboratory of Soil Science
Fytokou Road, 36 446 N. Ionia Magnisias
Greece

[2] Szent István University,
Department of Soil Science and Agro chemistry
Godollo,Hungary

3 Faculty of Agricultural Technology, Department of Floriculture
Arta, Greece

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Presenting author email: egol@uth.gr

Abstract

In two soils from central Greece a pot experiment was conducted with the addition of zeolite and compost (based on Posidonia Oceanica leaves) (with different mixtures between them) at 5% addition rates (calculated on a soil dry weight basis).

Three types of tobacco plants (Burley, Virginia and Oriental) were cultivated by using the soils along with the amendments, and the Cd concentration in tobacco leaves was measured at 1st, 2nd and 3rd priming each. The pot experiment was conducted on both soils with zeolite and compost (using different mixtures) at 5% addition rates (calculated on a soil dry weight basis). An industrial (Compost Hellas®) compost, based by from the leaves of beached Posidonia Oceanica (from Kefalonia island) as a matrix, was also used for the cultivation of tobacco plants.

We found that the addition of zeolite in the soil1 leaded to high reduction of Cd concentration in all the three types of tobacco studied than the control soil1. The addition of zeolite seemed to reduce water-soluble, as well as, DTPA- extractable Cd concentrations, as the concentrations of Cd extracted in all the other treatments well higher (statistically significant) than those obtained by the use of zeolite in soil 1. The addition of zeolite in 5% (calculated on a soil1 dry weight basis) caused 58,3% reduction of Cd concentration in Burley, 22,2% in Virginia and 28,6% in Oriental tobacco plants.

If we take into account the different rates of zeolite and compost used as amendments in soil 1, we can easily come to a conclusion that the most effective amendment in soil 1 is a mixture consisting of 20% compost and 80% zeolite as the use of this mixture leaded to 61, 7% reduction of Cd concentration in Burley, 32,2% in Virginia and 41,4% in Oriental tobacco plants.

In the case of soil 2, which had almost twice Cd concentrations from the soil 1, the use of compost seemed to be more effective in reducing Cd concentration from al the three types of tobacco studied. The addition of compost (5%) on a soil 2 caused 51,2% reduction of Cd concentration in Burley, 46,9% in Virginia and 56,5% in Oriental tobacco plants, while a mixture consisting of 20% zeolite and 80% compost leaded to 39,2% reduction of Cd concentration in Burley, 45,9% in Virginia

and 44,1% in Oriental tobacco leaves. In all cases studied, both in soils 1 and 2, Cd concentration was higher in Burley tobacco leaves.

In the first soil the use of a mixture containing 80% zeolite and 20% compost caused higher reduction on Cd concentration, while in the second soil the addition of compost seemed to be the most effective ameliorative, as far as Cd concentration reduction is concern, in all types of tobacco studied.

Introduction

Among environmental pollutants, heavy metals have been the subject of particular attention because of their long-standing toxicity when exceeding specific thresholds. The environmental pollution of soils directly influences human health since they have excellent ecological transference potential (Kabata-Pendias and Pendias 1992). Especially cadmium is harmful to humans, animals and tend to bio-accumulate in the food chain. This metal can be associated with several reactive materials and may exist in various forms that reflect their solubility and availability to plants (Alloway 1990).

Tobacco (Nicotiana tabacum) can accumulate Cd at relatively high levels compared to other species and concentrations in field-grown tobacco leaves usually range from <0.5 to 5 mg Cd /kg dry matter, although higher values can also be found (Lugon-Moulin et al., 2004).

Amendments or ameliorants have long been used to improve the quality of agricultural soils (organic matter, lime) (Gworek B 1992). When dealing with mitigation of metal uptake however, most of the research has focused on remediation of cadmium contaminated soils in order to re-establish a vegetation cover and subsequently reduce wind and water erosion and contamination of the surroundings (Cheng and Hseu 2002). Different materials, natural or synthetic, organic or inorganic, have been tested with success to reduce Cd availability to plants. These materials include phosphate (P) compounds like rock phosphate iron (Fe) and manganese (Mn) oxides and oxyhydroxides, and Fe/Mn-bearing amendments organic amendments inorganic clay materials including micas (illites), vermiculites, 2:1 phyllosilicates modified or not, zeolites and sepiolite (Keller et al. 2005). Zeolites constitute an important class of natural and synthetic aluminosilicate crystalline microporous materials (Haidouti 1997, James and Sampath 1999). Current technologies for soil remediation are time consuming or too expensive. Therefore, it is imperative to develop techniques that can treat and stabilize contaminants in situ in an efficient and cost effective manner.

Compost obtained from a bio-oxidizing transformation process of selected organic wastes can be used as an amendment for soils. Particularly the disposal of the annual accumulation of Posidonia oceanica on the beaches of the Mediterranean can be considered refuse, and at present they are transported to waste dumps, with the resulting loss of this enormous mass of organic material. It, therefore, seems of value to suggest an alternative system for recycling this waste in a way that satisfies the most recent U.E. directives (Castaldi and Melis, 2004).

Posidonia oceanica is one of the most abundant marine phanerogams in Greece (Malea et al. 1994) and in Sardinia, or middle Adriatic sea (Kljakovic-Gaspic et al. 2004). Also, it is used as a bioindicator of water quality monitoring (Richir et al, 2015). In chemical terms, can be compared to other vegetal waste biomass. It is particularly rich in structural carbohydrates (C/N ratio>65%) and thus suitable to use as a growing medium and to combine in the right proportions with mainly nitric natural residues such as sewage sludge, a potentially compostable waste material (Castaldi and Melis, 2004). Also, it influences the geomorphology of sandy shores and dead leaves are frequently thrown up on beache forming “banquettes” which protect them from erosion (Karyotis et al, 2006).

The principal aim of the present work was to test whether a type of amendments or a mixture of them, were efficient at reducing Cd concentration in different types of tobacco leaves of plants grown in contaminated with Cd agricultural soils. The ratio of the amendments used that consults to higher reduction of Cd concentrations in tobacco plants was, also, of interest and discussed.

Materials and Methods

Two soils were collected from central Greece, Anhialos area (soil 1) and Industrial Volos area (soil 2). Soil 1 is an agricultural soil regularly used for tobacco production. Soil 2 is an industrial soil, as a result, is heavily contaminated with Cadmium (Table1).

The zeolite sample used for experimental work was a homogenised sample, consisting of specimens obtained from different sites of the deposit. The sample was sieved to obtain the l-2 mm grain size fraction. The powder-XRD analysis (using standard mixtures of the minerals) indicated that the final material used contained 61% HEU-type zeolite, 18% SiO,-phases (cristobalite, quartz), 8% feldspars (alkali-feldspars, plagioclase) and 13% phyllosilicates (micas, clay minerals) (Haidouti 1997). An industrial (Compost Hellas®) compost, based by from the leaves of beached Posidonia Oceanica (from Kefalonia island) as a matrix, was also used for the cultivation of tobacco plants.

The pot experiment was conducted on both soils with zeolite and compost (with different mixtures between them) at 5% addition rates (calculated on a soil dry weight basis) (Keller et al. 2005, Haidouti 1997). The pots were filled with 4 kg of soil 1 and 3.7 kg of soil 2 (difference due to different initial moisture content). All treatments were performed in triplicates. Three control pots per soil were also set up without amendment. Three different tobacco types were used for cultivation: Burley, Virginia and Oriental (n=9 for each of them). Seeds were sown in a metal-free substrate (peat or compost). When the plants had developed three pairs of leaves, they were transferred to the pots.

Soil properties were measured (Page et al. 1982) such as clay content (%), organic matter (Walkley-Black method), pH (1:1) and electrical conductivity (1:1). Plant available fractions of metals were determined by using diethylene-triamine-pentacetic acid (DTPA) buffered at pH 7.3 (Lindsay and Norvell 1978). Total concentration of metals was determined using the Aqua Regia (HCl-HNO3, 3:1) extraction method (ISO/DIS 11466 1994) after digestion at 180o C for 2 h. All reagents were of analytical grade (Merck, Germany). The stock solutions of metals (1000 mg/mL) were prepared from “titrisol” Merck.

Cd concentrations were determined by atomic absorption spectrophotometry (AAS) using flame (F-AAS) or the Graphite Furnace (GF) technique (Lajunen 1992). Deuterium background corrections were used in the analysis of Cd with the GF-AAS followed the standard Methods of AOAC (1984). Certified Reference Material (CRM) (No 141R, calcareous loam soil) by BCR (Community Bureau of Reference) was also analyzed for the verification of the accuracy of Cd determination in soils. Recovery values were calculated as the ratio of the BCR results to those of the Aqua Regia digestion and ranged from 95 to 101 %. The detection limits based on three times the standard deviation of the blank (n=10), were found between 0.08 μg L-1 (GF-AAS) respectively.

Three tobacco leaves from each priming, with two replicates, were selected from plants. First priming included lower leaves and third priming upper leaves.. The leaves were washed to remove any adhering soil particles and rinsed with distilled water. After that leaf samples were placed in paper bags, dried at 75 o C for 12 hours and ground using a mortar and pestle. The extraction method procedure described by He and Singh (1994a) was followed. Then they were analyzed for metals concentration as previously described. Appropriate blanks were included in all extractions.

The comparison of Cd contents both in soil extractants and in tobacco leaves of the study was carried out using the t-test. Results from two replicates were averaged prior to statistical analyses, which they obtained using SPSS® (Statistic Program for Social Sciences) for Windows.

Results and Discussion

Table 1 shows the values of chemical and physical properties of the soil samples. Both soils have organic carbon content in the range usually found for Greek agricultural soils and clay content that is not statistically different between them. The first soil characteristics are favourable to low metal availability to tobacco plants and H2O, DTPA-extractable, as well as, total Cd concentrations, are all lower than the respective Cd concentrations of the second soil.

Table 1. Chemical and physical properties of soil samples

	Soil 1	Soil 2
pH	7.6	5.9
Organic C (%)	1.9	1.6
Clay (%)	18	16
Slit (%)	39	32
Sand (%)	43	54
H_2O-extractable Cd (mg/kg dry soil)	0.5	1.1
DTPA-extractable Cd (mg/kg dry soil)	1.8	2.9
Total Cd (mg/kg dry soil)	2.4	5.7

In Fiqure 1 the percentage (%) of Cd concentration by using different exctractants in soil 1 is illustrated.

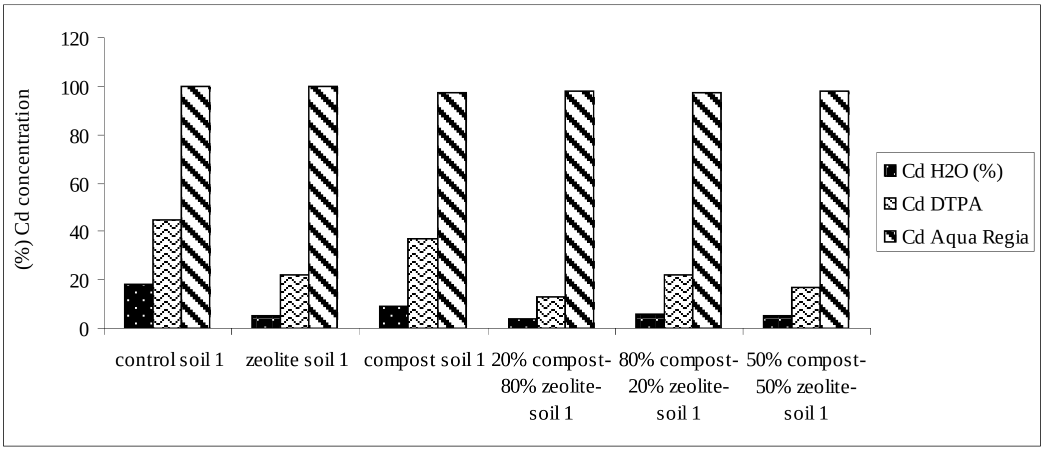

Figure 1. Cd fractions extracted from soil 1

The concentration of water soluble Cd, as well as the available to plants concentration of Cd (extracted with DTPA solution) were in all treatments lower than the control sample (soil 1 without any additions). The addition of zeolite seemed to reduce water-soluble, as well as, DTPA- extractable Cd concentrations, as the concentrations of Cd extracted in all the other treatments well higher (statistically significant) than those obtained by the use of zeolite in soil 1.

In Fiqure 2 the percentage (%) of Cd concentration by using different exctractants in soil 2 is illustrated.

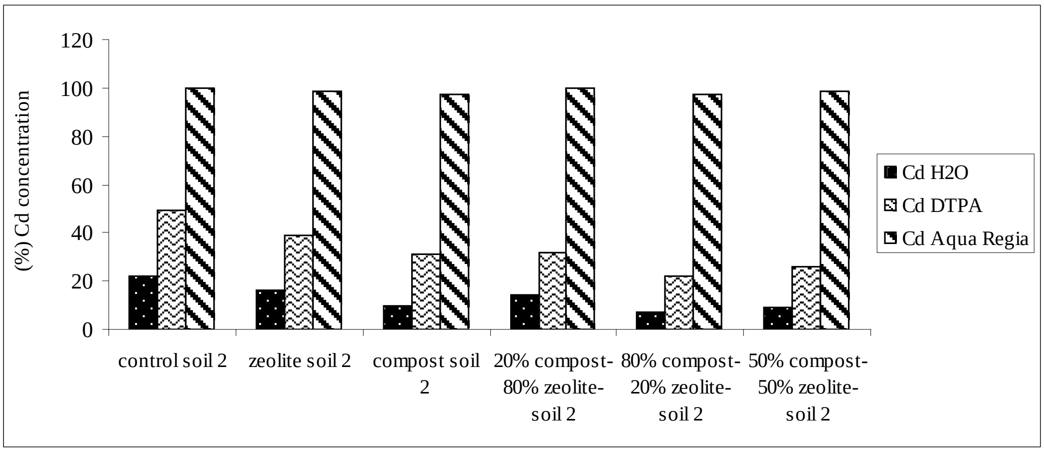

Figure 2. Cd fractions extracted from soil 2

As it was observed in soil 1, the amendments caused a remarkable reduction to water-soluble and available to plants Cd concentration. The use of compost as an amendment to soil 2 caused lower Cd concentrations rather than the use of zeolite, in contrary to soil 1.

Total Cd concentrations (extracted by the use of Aqua Regia mixture) were not statistically different (nor in 0.01 neither in the 0.05 levels of probability). On the other hand in all cases that the compost was used as an amendment both in soil 1 and 2, with or without zeolite, the total Cd concentration was lower than the Cd concentrations obtained without the addition of compost. When the compost is added to soils 1 and 2, the addition of perchloric acid ($HClO4$) would be more proper in order to extract total amounts of cadmium (ISO/DIS 11466 1994).

In Figures 3 and 4 Cd concentrations of a mixture of equal proportions of 1st, 2nd and 3rd priming of Burley, Virginia and Oriental tobacco plants, grown in soils 1 and 2, respectively, are illustrated. In previous studies concerning about the accumulation of Cd in tobacco plants grown in central Greece the 1st priming seamed to accumulate greater amounts of Cd (Golia et al. 2007), but in some other researches Cd concentrations were higher in the middle position or upper leaves (Lugon-Moulin et al. 2004). In order to have more real able conclusion about Cd concentration reduction in tobacco leaves by using the above mentioned amendments we analyze a mixture of the three primings, resuming that a mixture of the three positions leaves are being used for the cigarettes.

It is obvious (Figure 1) that the addition of zeolite in the soil1 leaded to high reduction of Cd concentration in all the three types of tobacco studied than the control soil1. The addition of zeolite in 5% (calculated on a soil1 dry weight basis) caused 58,3% reduction of Cd concentration in Burley, 22,2% in Virginia and 28,6% in Oriental tobacco plants. The use of zeolite in order to reduce metal availability to plants has been used from a lot of researchers (Gworek B 1992, Haidouti 1997, Keller et al. 2005).

If we take into account the different rates of zeolite and compost used as amendments in soil 1, we can easily come to a conclusion that the most effective amendment in soil 1 is a mixture consisting of 20% compost and 80% zeolite as the use of this mixture leaded to 61,7% reduction of Cd concentration in Burley, 32,2% in Virginia and 41,4% in Oriental tobacco plants. The high pH value of soil 1 coincided well with the efficiency of Cd reducing in the extractability of the soils and uptake of the plants in each treatment (Cheng and Hseu 2002).

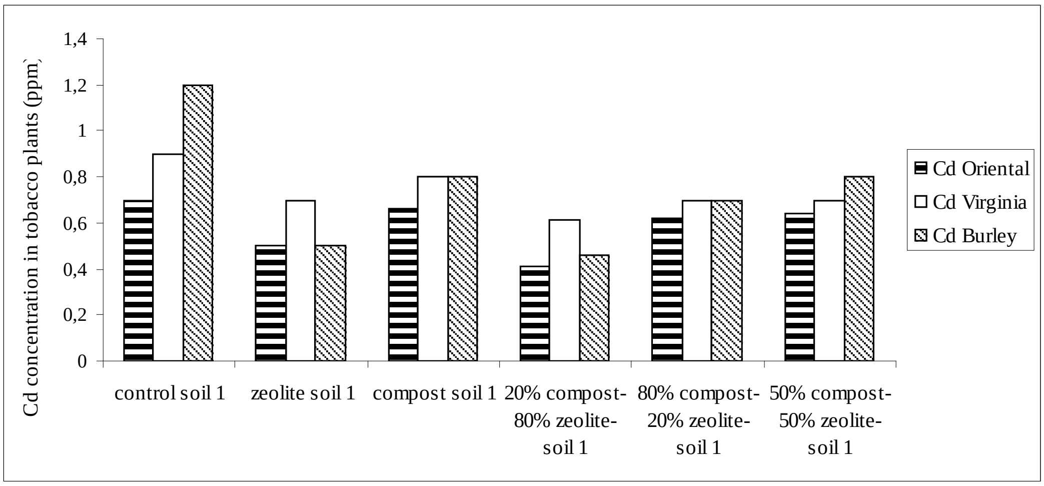

Figure 3. Cd concentrations in the three types of tobacco grown in soil sample 1

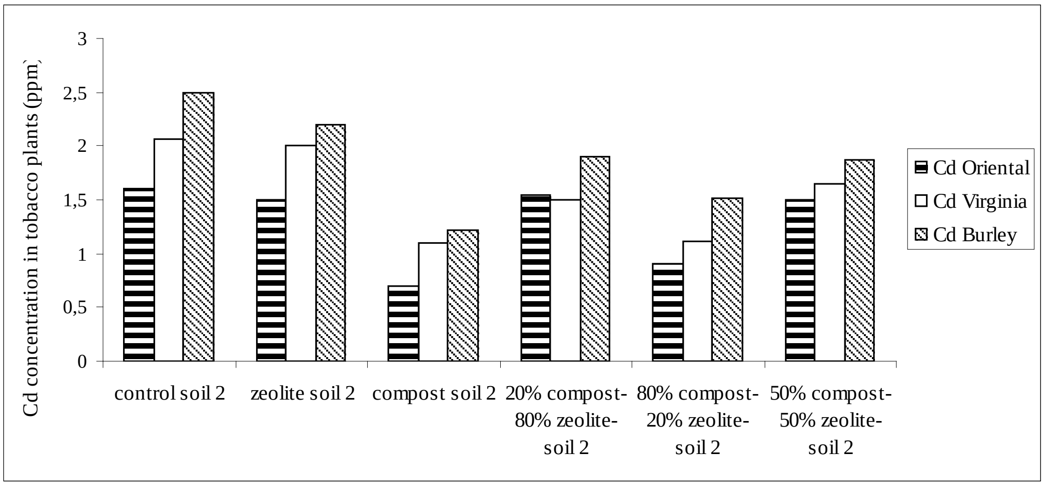

Figure 4. Cd concentrations in the three types of tobacco grown in soil sample 2

When zeolite or compost (with different rates between them) were added in soil 2 in 5% (calculated on a soil 2 dry weight basis) (Figure 4), in all cases studied Cd concentration in tobacco leaves were lower than the control soil 2 (without any amendment addition).

In the case of soil 2, which had almost twice Cd concentrations from the soil 1, the use of compost seamed to be more effective in reducing Cd concentration from al the three types of tobacco studied. The addition of compost (5%) on a soil 2 caused 51,2% reduction of Cd concentration in Burley, 46,9% in Virginia and 56,5% in Oriental tobacco plants, while a mixture consisting of 20% zeolite and 80% compost leaded to 39,2% reduction of Cd concentration in Burley, 45,9% in Virginia and 44,1% in Oriental tobacco leaves. In all cases studied, both in soils 1 and 2, Cd concentration was higher in Burley tobacco leaves (Golia et al. 2007).

In Table 2 the correlation coefficients among Cd concentration in the three primings of the Oriental tobacco type cultivated in soils 1 and 2, with 5% addition rates with the mixtures 20% compost-80% zeolite and 100% compost, respectively, along with the soil parameters, water-soluble, plant available and total Cd concentrations are presented. These mixtures (20% compost-80% zeolite) and (100% compost) as 5% addition in soils 1 and 2 respectively, were chosen because they appeared to have better results as soils amendments, reducing Cd concentration in tobacco leaves, more than the other mixtures did. The significant correlation between soils and amendments underlines the necessity to choose the amendment according to the soil type (Keller et al. 2005).

Correlation coefficients in Virginia and Burley tobacco were lower and in some cases not significant neither in 0.05 nor in 0.01 level (Pearson Correlation, 2- tailed), so they are not presented.

Table 2. Correlation coefficients among Oriental tobacco type cultivated in soils 1 and 2 and soil parameters and Cd concentrations in soils

	Cd_{s1}-1st	Cd_{s1}-2nd	Cd_{s1}-3rd	Cd_{s2}-1st	Cd_{s2}-2nd	Cd_{s2}-3rd
pH	-0,744**	-0,634**	-0,711**	-0,794**	-0,634**	-0,455**
EC	-0,345*	-0,422*	-0,294*	-0,544*	-0,348*	-0,546*
OM	0,655*	0,522*	0,463*	0,778*	0,599*	0,622*
Clay	0,256*	0,336	0,358	0,526*	0,299	0,325*
Cd_{H2O}	0,366*	0,355*	0,365*	0,299*	0,411*	0,362*
Cd_{DTPA}	0,526*	0,599*	0,536*	0,669*	0,635*	0,653*
$Cd_{Aqua\ Regia}$	0,229*	0,323	0,269	0,522*	0,266	0,294

** Correlation is significant at the 0.01 level (2-tailed),

* Correlation is significant at the 0.05 level (2-tailed)

Cd concentration in all the three Oriental tobacco primings correlated negatively with soil pH. This is well documented from the literature (Lugon-Moulin et al. 2004, Golia et al. 2007), as tobacco plants grown in acid soils usually accumulate higher Cd amounts, than in calcareous soils. The correlation was high even in the case of soil 1 (calcareous) indicated that the treatment with zeolite or compost caused also Cd accumulation in tobacco leaves when soil-amendment pH reducing. Organic matter content was highly correlated with Cd concentration of the 1st, 2nd and 3rd priming of Oriental tobacco leaves grown in the second soil as the addition of compost in soil 2 caused the higher reduction of Cd concentration in Oriental tobacco leaves. This is also well documented from the literature, as the plants or vegetables grown on the compost-based media showed reduced absorption level of potentially toxic metals (Mininni et al, 2014). Cd concentration in leaves was, also, positively correlated with plant available (extracted with DTPA) soil fraction, something that is expected for, as it is well documented from the literature (Alloway 1990; Kabata-Pendias and Pendias 1992). There was no significant correlation among Cd concentration in Oriental tobacco primings and total cadmium concentrations (extracted with Aqua Regia) neither in soil 1 nor in soil 2 studied.

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