

1 **The effect of manure, zeolite and soil ageing in the dynamics of hexavalent chromium**  
2 **in *Cichorium spinosum***

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8  
9 Abstract

10 Hexavalent chromium is a toxic anionic species, found in soils mainly due to anthropogenic  
11 activities. In this experiment we studied the effect of added Cr(VI) to *Cichorium spinosum*, a  
12 suspected tolerant wild edible vegetable. We added manure so that reduction of Cr(VI) to the  
13 inert species Cr(III) may be induced; this was compared with two other treatments representing  
14 possible mitigation techniques: addition of natural zeolite and soil ageing. Our aim was to  
15 compare these three Cr(VI) methods of decreasing Cr(VI) bioavailability. In a 60-day pot  
16 experiment we had 5 treatments replicated 5 times, as follows: Unamended control (treatment  
17 C), soil added with 100 mg kg<sup>-1</sup> Cr(VI) (treatment S), soil with 1% zeolite plus 100 mg kg<sup>-1</sup>  
18 Cr(VI) (treatment Z), soil with 1% manure plus 100 mg kg<sup>-1</sup> Cr(VI), and a soil added with 100  
19 mg kg<sup>-1</sup> Cr(VI) one year before the experiment (treatment AS, for “aged soil”). We found that  
20 at Z Cr(III) was the highest among the other treatments; Cr(VI) was the highest at S, while Z,  
21 M and AS had significantly lower concentrations than S (but higher than the control). In plant,  
22 at Z, S, and AS Cr(VI) concentrations were high and without differences, while at M Cr(VI)  
23 was significantly lower. Similar were the findings for plant Cr(III). This indicates that added  
24 manure did not decrease Cr(VI) availability to *C. spinosum* due to reduction to Cr(III), but  
25 rather due to the formation of organometallic complexes. We conclude that added manure was  
26 the best practice for mitigating Cr(VI) bioavailability.

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28 Keywords: Bioavailability, biomass, *Cichorium spinosum*

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30 1. Introduction

31 Hexavalent chromium is a highly toxic and easily mobilised trace element. In soil solution it is  
32 found in the anionic form of CrO<sub>4</sub><sup>2-</sup> or HCrO<sub>4</sub><sup>-</sup>. Due to the fact that the most important soil  
33 colloids bear negative charge on their surfaces, Cr(VI) is not capable of being retained by soil  
34 constituents. Thus it is highly mobile in the soil environment, and readily absorbed by plants  
35 [1]. Although Cr(VI) may be of natural origin, high concentrations are usually associated with

36 anthropogenic inputs. The plain of Assopos river, South Greece, is suspected of having high  
37 concentrations of Cr(VI) due to the deposition of wastes of certain industrial plants situated in  
38 that area [2]. In that same area, garden vegetables are cultivated and traded; thus fears of  
39 introduction of Cr(VI) into the human food chain are not unsubstantial.

40  
41 It is well known that Cr(VI), when added to soil, is eventually reduced over time to its trivalent  
42 form [3]. Cr(III) is a cationic species, found as  $\text{Cr}^{3+}$  in the soil solution; for this reason it is  
43 strongly retained by soil colloids, and is thus very slow in being absorbed by plants. Thus a  
44 possible Cr(VI) mitigation practice would be to allow sufficient soil ageing time after Cr(VI)  
45 addition to soil. This applies to a situation where Cr(VI) is not added continuously to soil. On  
46 the other hand, organic matter is known to have a positive effect in reducing Cr(VI) to Cr(III)  
47 in soil [4]. Thus another technique to mitigate Cr(VI) contamination would be to add organic  
48 materials (e.g., manure). Another way to decrease Cr(VI) to soil may also be the addition of  
49 positive-charge surfaces to soil. Such materials could attract Cr(VI) from the soil solution,  
50 decreasing thus their activity and their uptake by plants. Various materials have been  
51 suggested, with surface-modified zeolite being the most widely used [5]. However, there is a  
52 marked disadvantage in using modified zeolites: they are water-repellent and thus create  
53 hydrophobic films in soil that cause water stress to plants. Thus modified zeolite have rarely  
54 been used in growth experiments. Alternatively, natural zeolites could be used, despite the fact  
55 that it is negative-charged [6]. Although Cr(VI) is found in soil solution as an anion,  
56 application of zeolite to soil may retain Cr(VI) through physical entrapment in its pores.

57  
58 To our knowledge, there is no work that would compare those three Cr(VI) mitigation  
59 practices (soil ageing, addition of manure and addition of zeolite) to vegetables. Our test plant  
60 was *Cichorium spinosum*, a vegetable never tested before in a similar study. *C. spinosum* is a  
61 wild species that tolerate harsh environmental conditions. It is thus suspected that it could  
62 serve as an alternative cultivation in areas affected by Cr(VI) contamination. We thus wanted  
63 to test its tolerance to Cr(VI) exposure. The aim of this work was to test addition of manure,  
64 the addition of zeolite, and soil ageing as Cr(VI) mitigation practices in decreasing Cr(VI)  
65 contamination in a soil cultivated with *C. spinosum*.

## 66 67 2. Materials and Methods

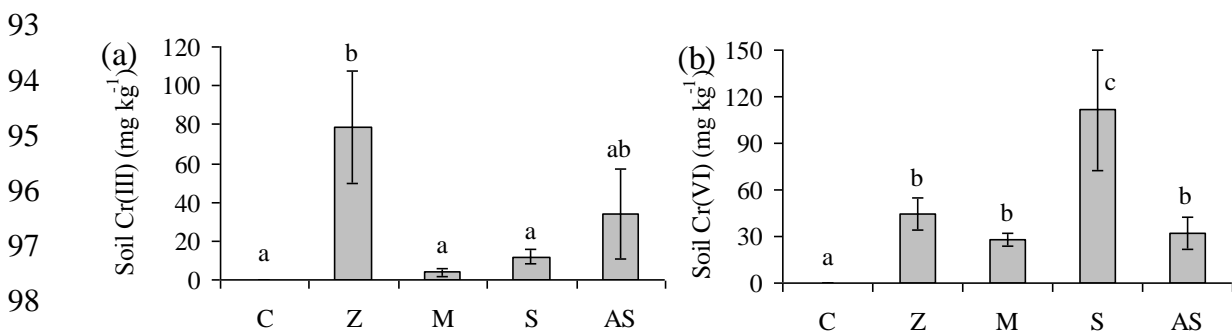
68 We studied a soil with organic C 1.30% and slightly alkaline (pH 7.5). We had the following  
69 treatments:

70 (a) Treatment C: Control soil, with no additions

- 71 (b) Treatment S: Soil added with 100 mg Cr(VI) kg<sup>-1</sup>  
 72 (c) Treatment Z: Soil added with 100 mg Cr(VI) kg<sup>-1</sup> and 1% w/w zeolite  
 73 (d) Treatment M: Soil added with 100 mg Cr(VI) kg<sup>-1</sup> and 1% dry farmyard manure  
 74 (e) Treatment AS: The same soil, amended one year before this experiment with 100 mg  
 75 Cr(VI) kg<sup>-1</sup> was used (named AS for “aged soil”).

76  
 77 These 5 treatments were replicated 5 times. We placed these treatments into 1-L capacity pots,  
 78 where we put 1 kg of each of the treatments, and *C. spinosum* was sowed; the pots were  
 79 watered regularly. We harvested the plants 60 days after sowing. After harvesting, soil samples  
 80 were taken, extracted and analysed for Cr(III) (with CaCl<sub>2</sub>-DTPA) and Cr(VI) (with 0.01 M  
 81 KH<sub>2</sub>PO<sub>4</sub>). Aerial biomass was also harvested, dried in a forced-draught oven until no further  
 82 weight loss was recorded, weighed, dry ashed, extracted with 20 mL 20% HCl and analysed  
 83 for Cr(III) and Cr(VI). For soil, DTPA-extracted Cr(III) was analysed with atomic absorption  
 84 spectrometry (AAS), and Cr(VI) colourimetrically with dithenyl-carbazide in a  
 85 spectrophotometer at 420 nm. For plant, we analysed the plant extracts with AAS for the  
 86 determination Cr(III) + Cr(VI), and also colourimetrically in spectrophotometer for the  
 87 determination of Cr(VI). The concentration of Cr(III) was then calculated as the difference  
 88 between AAS-analysed Cr(III)+Cr(VI) and spectrophotometer-analysed Cr(VI). The data were  
 89 analysed for statistical significance with one-way ANOVA; we used the statistical package  
 90 IBM SPSS Statistics v.21.0 for Windows (IBM corp.).

91  
 92 **3. Results and Discussion**



99 Fig. 1. Soil concentrations of (a) Cr(III) and (b) Cr(VI).  
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101 Although we did not find any Cr(VI) and Cr(III) in the unamended soil, the addition of the  
 102 metal resulted in a significant increase in soil Cr(III) levels. This increase was significant for  
 103 treatment Z (Fig. 1a). On the other hand, Cr(VI) increased significantly compared to the  
 104 control in all Cr(VI)-added treatments, with S having significantly higher concentrations  
 105 compared to Z, M, and AS (Fig. 1b). Bearing in mind that we added only Cr(VI) at a rate of  
 106 100 mg Cr(VI) kg<sup>-1</sup> soil, all Cr(III) we found was the result of the reduction of added Cr(VI).

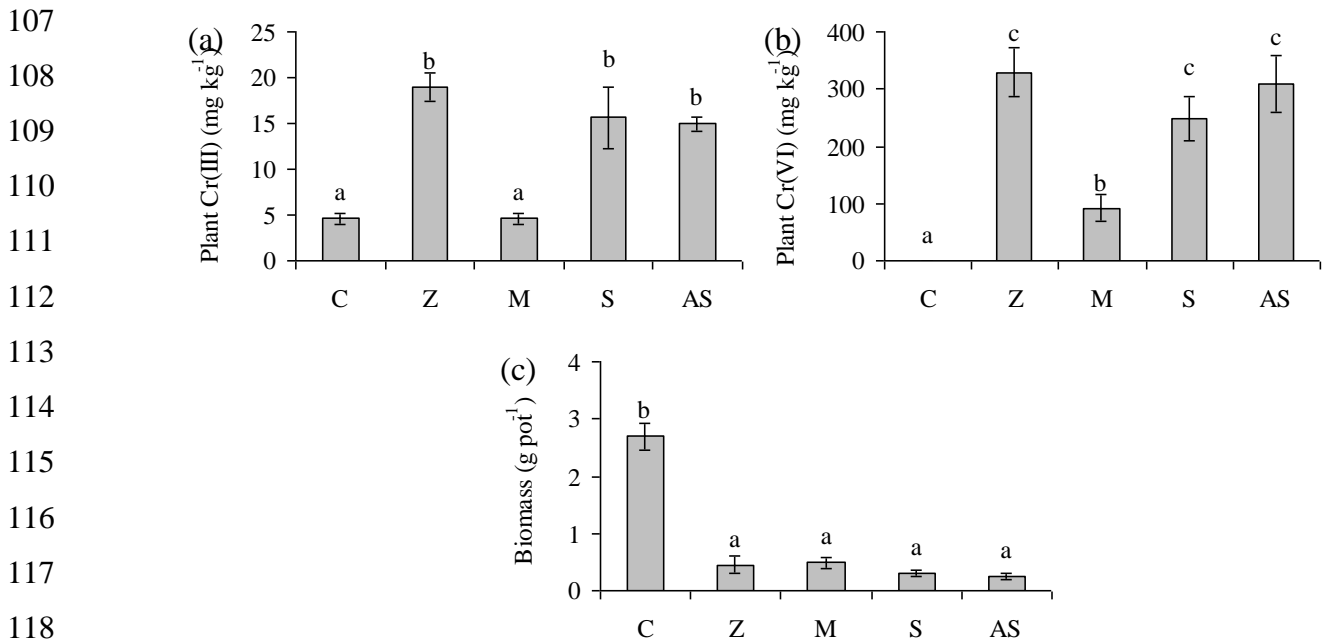
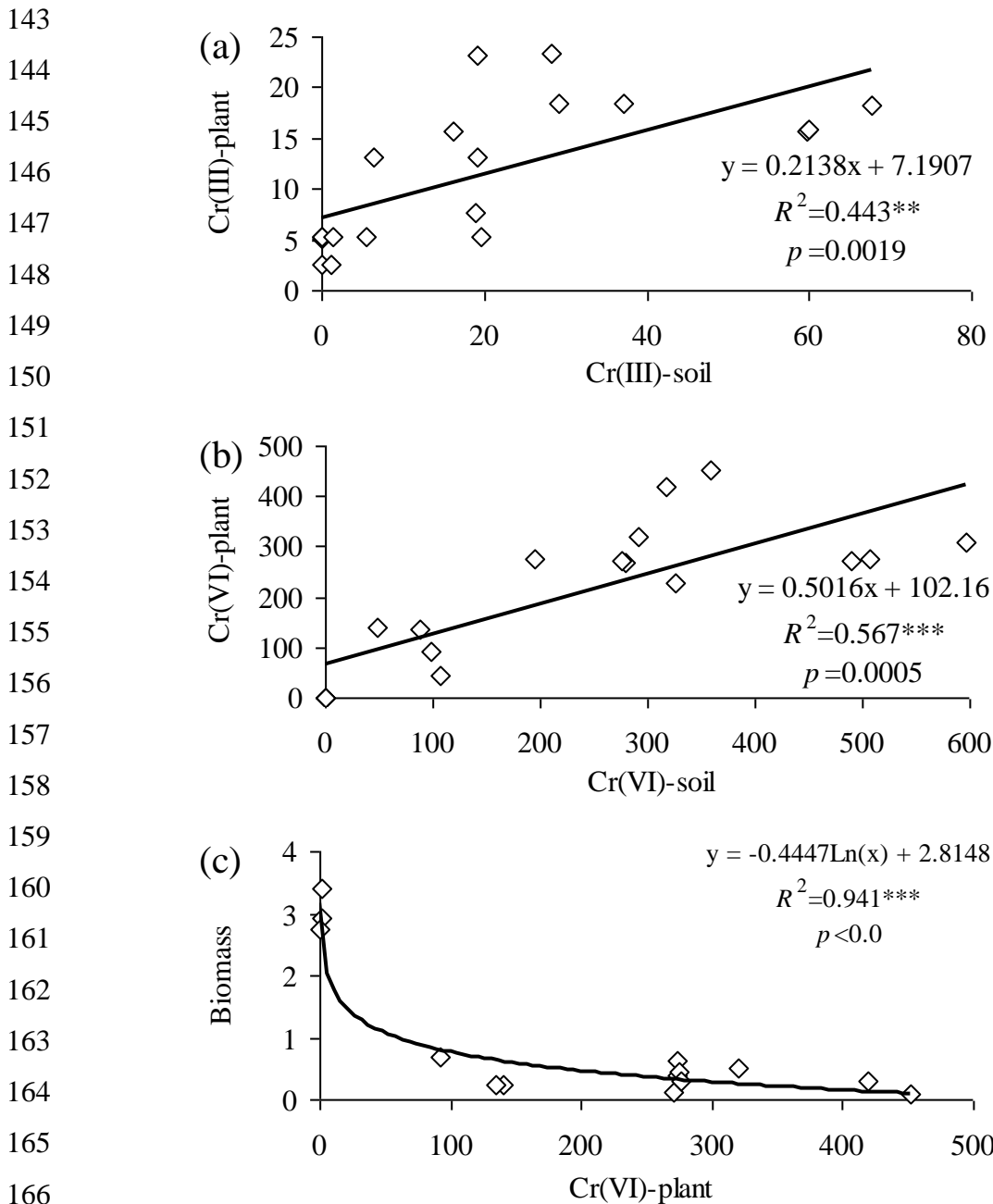


Fig. 2. Plant concentrations of (a) Cr(III) and (b) Cr(VI), as well as (c) aerial biomass.

Treatment Z seems to have induced more Cr reduction from valency VI to III than the other treatments. This may be associated with the porosity of zeolite; it is possible that formed Cr(III) at Z was trapped into the zeolite pores and released slowly, so that it was protected from being taken up by the test plant. On the other hand, Cr(VI) in S was similar to that initially added, indicating that no reduction to Cr(III) took place (unlike the findings by [7]); it also shows that it was the amendments of zeolite and manure along with ageing that caused the reduction of Cr(VI) in the Z, M, and AS treatments, respectively. In the treatments where we tested the three mitigation techniques, Cr(VI) levels were similar and without significant differences among them. While low Cr(VI) levels at M and AS were not unexpected, it is interesting that even at Z Cr(VI) was comparatively low. Added zeolite seems to have caused the reduction to Cr(III) and the decrease of Cr(VI) soil levels.

In plant, Cr(III) was detected at control in very low concentrations (<5 mg kg<sup>-1</sup>), and the levels at C were similar to those at M. The other 3 treatments (Z, A and AS) had significantly higher Cr(III) concentrations, but they were similar when compared among them (Fig. 2a). Cr(VI) was zero at C; among the Cr(VI)-added treatments, M had significantly lower concentration compared to the others, while Z, S, and AS had no differences among them (Fig. 2b). This shows that in all Cr(VI)-added treatments, uptake was similar among treatments, despite the differences in soil Cr(III) levels; exception was treatment M, which was lower. Likewise, irrespective of soil Cr(VI) levels, plant uptake was substantial at Z and AS, similar to that at S. This means that at plant level, zeolite and soil ageing did little to decrease Cr(VI) uptake compared to the unprotected soil (treatment S). Our results, if soil and plant data are taken into



167 Fig. 3. Correlation between (a) Cr(III) in soil and plant, (b) Cr(VI) in soil and plant, (c) Cr(VI) and biomass, and (d) organic C and % Cr(III)-over-Cr(VI) ratio in soil.  
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 169

170 consideration, suggest that zeolite enhanced the evolution of Cr(III) (as indicated in Fig. 1a),  
 171 but it could not bind the remaining Cr(VI), which was readily absorbed by plant. On the other  
 172 hand, soil ageing resulted in lower soil Cr(VI) levels (Fig. 1b), but it seems that the remaining  
 173 Cr(VI) was still highly available to plant and sufficient to accumulate at high concentrations in  
 174 plant [8]. The treatment that had the best efficiency in reducing plant uptake was M. At M  
 175 plant Cr(VI) was the lowest, excluding C, similar to soil Cr(VI) (although in soil the difference  
 176 of M compared Z and AS was not significant). Manure seems to have affected Cr(VI) in a  
 177 positive way. There two main mechanisms this may have occurred: (a) the accelerated  
 178 reduction of added Cr(VI) to Cr(III), and (b) the formation of organometallic complexes unable

179 to be absorbed by the plant. If the first mechanism was dominant, soil Cr(III) at M should be  
180 higher than at other treatments. Alternatively, the ratio of Cr(III)/Cr(VI) should be higher at M  
181 compared to other treatments, because higher ratio would indicate higher evolution of Cr(III)  
182 compared to Cr(VI) in soil. However, the Cr(III)-over-Cr(VI) ratio was similar across  
183 treatments (data not shown). Thus the possible reason is mechanism (b). Organometallic  
184 complexes, if of sufficiently high molecular weight, are incapable of being absorbed by plant,  
185 and this causes a reduction in Cr(VI) uptake. Indeed such complexes are reported to increase in  
186 soils where Cr(VI) and organic matter have been added [9].

187  
188 Dry plant biomass indicates Cr(VI)-induced toxicity (Fig. 2c): in all treatments plant weight  
189 decreased significantly compared to the control. Moreover, no differences among Z, M, S, and  
190 AS were found.

191  
192 In order to further investigate the various effects of added Cr(VI) to soil, we performed  
193 correlation analyses. As expected, soil Cr(III) was significantly correlated with plant Cr(III)  
194 (Fig. 3a), and so was Cr(VI) (Fig. 3b). Correlation analysis confirmed the adverse effect added  
195 Cr(VI) had on plant biomass: it showed that added Cr(VI) significantly reduced plant biomass.

196  
197 4. Conclusions  
198 We conclude that the addition of manure is the best practice to minimize the toxic effects of  
199 added Cr(VI) to *C. spinosum*.

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