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Phytoremediation of Antimony Contaminated Soils Using the Bioenergy Plant *Cynara cardunculus*

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Abstract

Antimony (Sb) is an emerging environmental pollutant due to its toxicity, persistence, and extensive industrial applications. Despite the growing need for sustainable remediation strategies, research on the potential of phytoremediation for Sb-contaminated soils remains limited. This study investigates the suitability of *Cynara cardunculus* (cardoon), a high-biomass bioenergy crop, for the remediation of Sb-polluted soils and evaluates the effect of Fe(II) supplementation on plant performance and Sb behavior. Pot experiments were conducted using soils amended with 10–40 mg Sb kg^{-1}, under treatments with and without Fe(II). In the absence of iron, cardoon showed high tolerance to Sb exposure, with no significant growth inhibition even at 40 mg Sb kg^{-1} after 30 days of cultivation. Iron addition significantly enhanced plant growth, resulting in a 2.3-fold increase in above-ground biomass compared with non-amended soils under the 20 mg Sb kg^{-1} treatment after 45 days of cultivation. Sb accumulation was mainly restricted to the root system, indicating limited phytoextraction capacity. However, the species demonstrated strong phytostabilization potential, as the presence of plants reduced the water-soluble Sb fraction in soil by up to 50% compared with unplanted controls. These results suggest that *C. cardunculus* is a promising candidate for phytostabilization of Sb-contaminated soils. Its combined use with iron amendments may enhance biomass production and support integrated soil remediation and bioenergy production strategies.

Keywords: *Cynara cardunculus*; antimony (Sb); phytostabilization; metal tolerance; bioenergy crops; iron amendment; soil contamination

1. Introduction

Antimony (Sb) has recently been recognized as an emerging contaminant of increasing environmental concern owing to its extensive use in flame retardants, alloys, batteries, semiconductors, and military applications. Continuous mining, ore processing, smelting, and industrial activities have resulted in increasing Sb contamination of soils worldwide. Because Sb is toxic, potentially mobile, and frequently co-occurs with arsenic in mining environments, there is an increasing demand for sustainable remediation technologies capable of reducing its environmental risk. Beyond industrial uses, antimony compounds are employed in the medical treatment of diseases such as leishmaniasis and bilharziasis [1,2].

Antimony exists primarily in oxidation states Sb(III) and Sb(V) in the environment, with Sb(III) generally exhibiting greater mobility and toxicity. In nature, more than 100 primary and secondary antimony minerals have been identified [1,2]. Most primary Sb minerals occur as sparingly soluble sulfides, among which stibnite (Sb_2S_3) is the most

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important. Under oxygen-rich conditions, primary minerals undergo weathering to form secondary minerals, such as the oxides senarmontite (cubic Sb_2O_3), valentinite (orthorhombic Sb_2O_3), and stibiconite (Sb_3O_6OH), as well as iron-oxide compounds including schafarzikite ($FeSb_2O_4$) and tripuhyite ($FeSbO_4$) [3,4].

The estimated average concentration of antimony in the Earth's crust is approximately 0.2–0.3 mg kg^{-1} [2]. The distribution of Sb concentrations in European soils has been investigated through a collaborative study involving Geological Surveys from 26 European countries [5]. The median concentration across 840 samples was 0.6 mg kg^{-1}, while the maximum measured concentration was 31.1 mg kg^{-1} in France. In Greece, 40 soil samples were analyzed, with Sb concentrations ranging from 0.11 to 21.5 mg kg^{-1} and a median value of 0.84 mg kg^{-1}. Argyraki and Kelepertzis [6] investigated the geochemistry of soils in the wider urban area of Athens (~220 km^2) by collecting 238 samples. The concentration of Sb ranged between 0.1 and 41.7 mg kg^{-1} and the median value was 1.7 mg kg^{-1}.

Among all anthropogenic activities, mining and metallurgical operations are considered the most significant sources of antimony contamination in soils and natural waters. Zhao et al. [7] compiled available global data on Sb concentrations in soils from mining and metallurgical areas. In most countries, the average Sb concentration exceeds the guideline value of 36 mg kg^{-1} recommended by the World Health Organization, apart from Turkey and North Macedonia. In European mining soils, Sb concentrations range from 17.5 mg kg^{-1} to 1378 mg kg^{-1} in North Macedonia and Slovakia, respectively.

In Asia, Sb concentrations range from 8.53 mg kg^{-1} to 2542 mg kg^{-1} in Turkey and Japan, respectively. In Oceania, extremely high Sb concentrations were reported, ranging from 3626 mg kg^{-1} to 80,200 mg kg^{-1} in Australia and New Zealand, respectively. High Sb concentrations were also observed in African soils, particularly in Algeria, with an average value of 13,021 mg kg^{-1}. In South America, measurements were available only for Bolivia, where an Sb concentration of 37.4 mg kg^{-1} was reported, which is close to the recommended guideline value. In conclusion, Sb-contaminated soils resulting from mining and metallurgical activities are found worldwide, with Oceania ranking first in terms of contamination severity, followed by Europe, Asia, and South America, respectively [7].

Antimony pollution of soil and waterbodies poses serious ecological and health challenges, and effective remediation approaches are urgently needed. For toxic elements such as antimony, soil remediation strategies aim either at the physical removal of the contaminant from the soil or at its stabilization, i.e., immobilization through binding to solid phases or chemical structures that reduce its solubility and bioavailability. Various soil washing and extraction techniques have been explored for the removal of Sb from contaminated soils. Tokunaga et al. [8] evaluated the effectiveness of several inorganic acids (HCl, H_2SO_4, H_3PO_4, and HNO_3), organic acids (citric and tartaric acid), and chelating agents (EDTA and DTPA). The treatment was applied to three soils collected from an area affected by metal recycling activities, where contamination was primarily associated with Pb, but also with other metals and metalloids, including Zn, Cu, As, Sb, and Se. Sb concentrations in these soils ranged from 5.9 to 29.1 mg kg^{-1}, whereas the reference uncontaminated soil contained 0.44 mg kg^{-1}. The highest extraction yields, approximately 10–15%, were achieved using sulfuric, phosphoric, and citric acids. Guemiza et al. [9] applied soil washing to soil collected from a military shooting range in Canada contaminated with Pb, Cu, Zn, and Sb, where Sb concentrations were approximately 370 mg kg^{-1}. The treatment involved three sequential washing steps using a solution of 0.125 M H_2SO_4 and 4 M NaCl. Sb removal efficiencies ranged from 43.8% to 59.6%. Tan et al. [10] investigated the effectiveness of six different extracting agents for the simultaneous removal of Sb and As from soils collected from an antimony mining area. Three soils exhibiting low, moderate, and high contamination levels were examined, with Sb concentrations of 145, 720, and 6876 mg kg^{-1},

respectively, and corresponding As concentrations of 18, 103, and 380 mg kg^{-1}, respectively. The effectiveness of the extractants followed the order citric acid > tartaric acid > EDTA > HCl > Na_2HPO_4 > $CaCl_2$. Citric acid enabled simultaneous removal of Sb and As, achieving extraction efficiencies of up to 24% and 41%, respectively. Li et al. [11] conducted extraction experiments on soil artificially contaminated with Sb(V) to a final concentration of 400 mg kg^{-1}. Initially, the effectiveness of ten extractants was evaluated, including oxalic acid, acetic acid, tartaric acid, malic acid, citric acid, etidronic acid (HEDP), oxalic acid, tetrasodium glutamate diacetate (GLDA), EDTA, and KH_2PO_4. The maximum extraction efficiency (69%) was achieved using acetic acid, whereas extraction efficiencies obtained with the other acids ranged between 43% and 50%.

In stabilization approaches, various amendments are added to soils to reduce contaminant solubility and bioavailability [12]. Two major classes of amendments have been extensively studied: (a) biochars (BCs) and (b) composts derived from green waste (GWC) or municipal solid waste (MSWC). In most cases, these studies involved soils with polymetallic contamination, where Sb was not the primary pollutant.

Conventional remediation techniques such as soil excavation, chemical immobilization, and physicochemical treatment often entail high costs, substantial energy consumption, and secondary environmental impacts. As a result, sustainable remediation strategies have been increasingly investigated. Among these, phytoremediation—defined as the use of plants to reduce, remove, stabilize, or transform specific pollutants in soils and water—has emerged as a cost-effective and environmentally benign alternative for metal-contaminated sites. Phytoremediation encompasses several strategies, including phytoextraction, phytostabilization, rhizofiltration, phytodegradation, rhizodegradation, and phytovolatilization [13]. Metal removal is achieved mainly through phytoextraction, whereas phytostabilization aims to immobilize contaminants within the soil. Rhizofiltration is applied for the treatment of contaminated waters, primarily targeting inorganic pollutants but, in certain cases, also pathogenic microorganisms and viruses.

Compared with other potentially toxic elements, phytoremediation of Sb-contaminated soils remains poorly investigated. In particular, studies evaluating high-biomass bioenergy crops for Sb phytostabilization are extremely limited. Although antimony (Sb) is not an essential element for living organisms, several plant species have been reported to accumulate relatively high Sb concentrations in their roots, stems, leaves, and even flowers. In most cases, Sb preferentially accumulates in root tissues; however, partial translocation to aboveground organs has also been observed. This behavior raises concerns regarding food safety, as the consumption of edible plants cultivated in Sb-contaminated soils may pose risks to human and animal health. Plant Sb uptake is strongly influenced by its phytoavailability, soil properties, and Sb speciation. In soils, Sb occurs mainly in two oxidation states, trivalent Sb(III) and pentavalent Sb(V), with Sb(III) being considerably more toxic to plants [1,2,14,15]. Despite high total Sb concentrations in contaminated soils, only a small fraction is typically bioavailable and absorbed by plants [2,16].

Antimony accumulation varies significantly depending on plant species, growth location, and substrate conditions. Elevated Sb concentrations in plant tissues are most frequently observed in mining-affected areas, where soil contamination levels are also high. For example, Okkenhaug et al. [17] investigated Sb uptake by native plants in the biggest actively mining Sb region in the world in Xikuangshan, China. The highest concentration in the above ground tissues was measured in the shoots of *Boehmeria nivea* (L.) Gaudich, 4029 mg Sb kg^{-1}. The concentration in the soil substrate was close to 12,000 mg Sb kg^{-1} (453 mg Sb kg^{-1} water extractable fraction). High levels of Sb were also measured in the shoots of *Sedum lineare*, 3092 mg Sb kg^{-1}, collected from a mining area in Southwest China, with 1732 mg Sb kg^{-1} in the soil substrate [18]. Baroni et al. [19] investigated Sb uptake by

Achillea ageratum L. cultivated in a highly contaminated soil from an abandon mining area in Tuscany (>9000 mg Sb kg^{-1} total concentration, 793 mg Sb kg^{-1} extractable fraction). The plant accumulated 367 mg Sb kg^{-1} in leaves and 1105 mg Sb kg^{-1} in inflorescences.

Despite their high phytoaccumulation capacity, the native plants are not appropriate for use in phytoextraction projects, because the production of harvestable biomass is usually very low. The uptake of Sb by high biomass productive plants has been tested for the case of *Zea mays*, *Helianthus annus*, *Secale cereale*, *Ipomoea aquatica*, *Acorus calamus*, *Tamarix smyrnensis*, *Nerium oleander,* etc. (references in [2,20]). In all these plants, there was preferential accumulation of Sb in the roots, whereas the translocation of the element to the above ground harvestable parts was very limited.

It is noted that most Sb phytoremediation studies are oriented towards the principles of phytoextraction. Less attention has been paid to the phytostabilization option. Unlike other phytomanagement strategies, phytostabilization does not seek to remove heavy metals from contaminated sites; rather, it aims to immobilize them and thereby minimize risks to human health and the environment [21–23]. Phytostabilization in combination with several soil amendments has been tested for a range of heavy metals, such as As, Cd, Cr, Cu, Pb, Zn, etc., but to our knowledge there is no such attempt for the case of Sb.

Selection of appropriate plants is critical for successful phytoremediation. Ideal candidates combine rapid biomass production, tolerance to elevated contaminant concentrations, and an ability either to accumulate or stabilize in situ the target elements. The perennial Mediterranean species *Cynara cardunculus* L. (cardoon) has been identified as a promising candidate for the remediation of heavy metal-contaminated soils due to its high biomass yield, deep root system, and adaptability to marginal lands. Moreover *C. cardunculus* biomass can be valorized for biofuel production, thereby reducing remediation costs [24]. The potential application of *C. cardunculus* for the remediation of contaminated soils has previously been investigated for heavy metals such as Cd and Ni, as well as for metalloid arsenic [24–26]. However, no relevant studies addressing antimony (Sb) were identified in international literature. This knowledge gap, combined with the growing environmental concern regarding the dispersion and toxicity of antimony, constituted the primary rationale for selecting Sb as the target contaminant of this study. The use of perennial energy crops offers the opportunity to combine contaminant stabilization with biomass production, thereby improving the economic feasibility of phytomanagement. The potential utilization of *Cynara cardunculus* for the remediation of Sb-contaminated soils and the effect of simultaneous Fe(II) addition in alleviating Sb toxic effects and reducing its mobility were investigated in the present study. To the best of our knowledge, the potential of *Cynara cardunculus* for Sb phytostabilization and the influence of Fe amendment on its performance have not previously been investigated.

2. Materials and Methods

2.1. Experimental Design

A total of 13 experiments were conducted, and their description is given in Table 1. The investigated parameters included (i) the levels of Sb contamination (10, 20 and 40 mg kg^{-1}), (ii) the exposure time (15, 20 and 45 days) and (iii) the addition or not of Fe which was applied at a molar ratio 1.5:1 relative to antimony for all the levels of Sb contamination and all exposure times. The experiments were carried out applying artificial contamination on a typical soil sample from Attica region, Greece. The addition of 20 mg Sb per kg soil was applied as the central antimony (Sb) contamination level. Under the central conditions, the harvest time was 30 days after exposing the plants to contamination. Three control tests were also included in the experimental work, T11, T12 and T13. Test eleven (T11) was carried out without applying Sb contamination in the soil and with a plant which

was harvested at 30 days. Tests T12 and T13 were carried out without plants, but the soil was contaminated only with Sb (T12) or with Sb followed by Fe addition (T13). The objective of control trials T12 and T13 was to compare the soluble fraction of antimony, with and without plants, under identical conditions of contamination level, Fe addition, and exposure duration. All experiments were performed in triplicate. Thus, the overall experimental design included 39 pots in total—33 with plants (3 × 11) and 6 without plants (3 × 2). The placement of pots followed a randomized design to minimize positional bias.

Table 1. Description of the tests.

	Sb	Fe	Duration	Plant	Description
	mg kg^{-1}	mg kg^{-1}	Days		
T1	20	0	30	Yes	Central conditions (Sb and plant)
T2	20	13.8	30	Yes	Central conditions with Fe (Sb, Fe and plant)
T3	10	0	30	Yes	Effect of Sb contamination levels with plant
T4	10	6.9	30	Yes	Effect of Sb contamination levels with Fe addition and with plant
T5	40	0	30	Yes	Effect of Sb contamination levels with plant
T6	40	27.5	30	Yes	Effect of Sb contamination levels with Fe addition and with plant
T7	20	0	15	Yes	Effect of duration only with Sb and plant
T8	20	13.8	15	Yes	Effect of duration with Sb, Fe and plant
T9	20	0	45	Yes	Effect of duration only with Sb and plant
T10	20	13.8	45	Yes	Effect of duration with Sb, Fe and plant
T11	0	0	30	Yes	Control test without Sb addition and with plant
T12	20	0	30	No	Control test with Sb and without plant
T13	20	13.8	30	No	Control test with Sb and Fe addition and without plant

2.2. Soil

The soil used for the experiments originated from the region of Attica and was provided by a company operating in the field of earthmoving works. The soil was sieved at −2 mm and a representative sample of approximately 1 kg was separated using Jones Riffle splitters (LAARMANN Group B.V, Roermond, The Nederlands). The main characteristics of the soil were determined by applying standard procedures and are presented in Table 2. The particle size analysis was carried out by means of Bouyoucos hydrometer method [27]. Soil pH was determined in 1:1 soil/deionized water (DW) suspension with a Metrohm 827 pHmeter (Metrohm AG, Herisau Switzerland)and electric conductivity was measured with a WTW LF95 conductivity meter (Xylem Analytics Germany Sales GmbH & Co. Weilheim, Germany). The determination of Soil Organic Carbon (SOC) was carried out by measuring the weight loss after the ignition of a soil sample at 550 °C for 2 h [28]. The Loss of Ignition (LOI) was determined by applying ignition at 1000 °C for 1 h. The difference LOI-SOC provides an indication of the inorganic carbon content. The total concentration of main compounds and the trace elements content was measured by X-Ray Fluorescence (XRF) spectroscopy using Spectro XLab Pro (SPECTRO Analytical Instruments GmbH, Kleve, Germany). The water-soluble amount of Sb was determined by applying the European standard leachability procedure EN12457-2:2002 [29] (see Section 2.5).

As seen in Table 2, the texture of the soil belongs to the category of sandy clay loam, the content of organic carbon is relatively low, 2.9%, and the inorganic carbon content, as estimated by the difference LOI-SOC is close to 13%. Based on its physicochemical characteristics, the soil can be broadly classified as a Calcisol. The total content of antimony,

as determined by the XRF method, is 12.7 mg kg^{-1} and the water-soluble fraction is lower than 0.05 mg kg^{-1}.

Table 2. Physical and chemical properties of soil.

Parameter	Value
Size fractions (%)	
Sand	62
Silt	18
Clay	20
pH	8.2
EC (μS/cm)	6.15
Loss of Ignition, LOI (%)	15.9
Soil Organic Carbon, SOC (%)	2.9
Main components (%)	
SiO_2	39.5
CaO	18.4
Al_2O_3	11.5
Fe_2O_3	5.9
Total Sb (mg kg^{-1})	12.7
Water soluble Sb (mg kg^{-1})	<0.05

2.3. Application of Plants and Artificial Contamination of Soils

Seedlings of *Cynara cardunculus* were obtained from a nursery. At the time of acquisition, the seedlings were 60 days old from sowing. Transplantation into the experimental pots was conducted after an additional period of 60 days. Each 2 L pot was prepared by placing a base layer of pumice to ensure adequate drainage and aeration, followed by 1.0 kg of the soil and the seedling along with its original potting substrate. After transplantation, plants were maintained for a 14-day acclimation period and irrigated every 4–5 days. At the end of the acclimation period (plant age 134 days), artificial soil contamination was performed. Antimony (Sb) solutions were first applied to the designated pots, followed 4 days later by the addition of iron (Fe) solutions.

Antimony solutions were prepared using the $K_2Sb_2(C_4H_2O_6)_2 \cdot 3H_2O$ salt. Potassium antimony tartrate was selected as a highly water-soluble source of Sb(III) commonly used in laboratory studies. The potential influence of tartrate ligands on Sb behaviour is discussed in Section 4. The iron solutions were prepared using the $FeSO_4 \cdot 7H_2O$ salt. Fe(II) was selected instead of ferric iron to prevent acidic shifts in soil pH, as Fe(III) solutions exhibit pH values < 2.5, compared with approximately pH 5.5 for Fe(II). Stock solutions of appropriate concentrations were prepared to allow the application of 100 mL of solution per pot, ensuring the target pollutant concentration in the soil. Preliminary tests confirmed the absence of drainage losses at this volume. All solutions were applied dropwise using fine-nozzle laboratory bottles to achieve uniform distribution within the root zone. Subsequent irrigation followed the same procedure at intervals of 4–5 days.

2.4. Harvest and Analysis of Plants

Most plants were harvested 30 days after the addition of antimony. In tests T7 and T8 harvest took place after 15 days and in tests T9 and T10 after 45 days. The harvested plants

were separated into root systems and above-ground parts, washed carefully with tap water and finally with deionized water. Then, drying was applied at a mild temperature of 43 °C for 5 days, and their dry weight was determined by weighing on a precision balance.

Antimony (Sb) concentrations in plant tissues were determined by inductively coupled plasma mass spectrometry (ICP-MS). Samples were cut with a hand mill, finely ground in an agate mortar, and 0.5 g aliquots were microwave-digested (MILESTONE ETHOS) with 7 mL HNO_3 and 1 mL H_2O_2. The digests were quantitatively transferred to 50 mL volumetric flasks and diluted to volume with deionized water. To reduce the organic load below 20 mg L^{-1} prior to ICP-MS analysis, 10 mL of the primary digest was treated with 2 mL HNO_3 and 1 mL H_2O_2, heated for 2–3 h, evaporated nearly to dryness, and re-diluted to 50 mL. The method detection limit (MDL) for Sb in plant tissues was 2.5 mg kg^{-1}.

2.5. Determination of the Water-Soluble Antimony in Soils

The determination of the water-soluble fraction of antimony in soils was carried out by applying the standard European leachability test EN12457.02 [29]. The EN 12457.02 test involves the leaching of the sample with deionized water (DW) at a liquid to solid ratio, L/S = 10 L kg^{-1}. The duration of the test is 24 h and within this time it is assumed that equilibrium between the liquid and solid phases is achieved. The test was carried out by mixing 50 g of the solids tested with 500 mL DW in HDPE bottles, which were placed on a rotary shaker (10 rpm).

After the end of the leaching period, the suspended solids were allowed to settle for 15 ± 5 min. The samples were then filtered using a vacuum pump and 0.45 μm membrane filters. The volume of the filtrate and its physicochemical parameters (pH, conductivity) were measured, while the solution was acidified and analyzed for Sb using the ICP-MS method. The water-soluble content was expressed in mg kg^{-1} of dry sample, which was calculated considering that the liquid to solid ratio was equal to 10 L kg^{-1}. With this procedure the detection limit for the water-soluble Sb in soil was 0.05 mg kg^{-1}.

2.6. Calculation of Translocation and Bioconcentration Factors

The tendency for a substance to move from the roots to the aerial parts of the plant is described by the translocation factor, *TF*, which is defined as the ratio concentration in the shoots (C_{shoots}) versus concentration in the roots (C_{roots}): $TF = C_{shoots}/C_{roots}$.

The bioconcentration factor (*BCF*) provides a measure of the ability of a plant to accumulate a metal contaminant in the plant tissues. It is defined as the ratio of the concentration in the phytomass (C_{plant}) versus the concentration of the element in the soil (C_{soil}): $BCF = C_{plant}/C_{soil}$. In most publications, the *BCF* reports on the accumulation in the aerial harvestable parts, $BCF_{aerial} = C_{shoots}/C_{soil}$, and provides a criterion of the capacity of the plant to be used in phytoextraction remediation projects. When *BCF* reports on the accumulation in the roots, $BCF_{roots} = C_{roots}/C_{soil}$, the values reflect a possible capacity to be used in phytostabilization or rizofiltration projects.

Because the soil's native Sb (12.7 mg kg^{-1}) was geochemically stable and not water-soluble, the added Sb concentration was considered the environmentally relevant exposure concentration for the calculation of the *BCF*.

2.7. Statistical Analysis

All tests were carried out in triplicate. The results were first subjected to the Fligner–Killeen test to evaluate the homogeneity of variances and then analyzed using factorial analysis of variance (single and two factor ANOVA). The statistical analysis was performed using the Real Statistics Resource Pack. Significance was accepted at $p \leq 0.05$ level.

3. Results

3.1. Biomass Production

3.1.1. Effect of Addition Sb and Sb + Fe on Biomass Production

Figure 1 shows the plant mass produced in the tests carried out without any pollution (T11), with the addition of Sb 20 mg kg^{-1} (T1) and with the simultaneous addition of Sb and Fe, 20 and 13.8 mg kg^{-1}, respectively (T2). The harvest time was 30 days after the application of the pollution. As can be seen in the figure, the addition of Sb at this level of pollution had no negative impact on the aerial plant mass produced. The mean value of the dry weight of shoots biomass was 1.25 g in the control test, 1.36 g with the addition of Sb and 1.83 g with the simultaneous addition of Sb and Fe. The differences are considered as statistically non-significant ($p = 0.24$). In the root system, the mean root biomass increased from 0.83 g in the control treatment (T11) to 1.56 g in the Sb-amended treatment (T1). Although this represents an almost two-fold increase, the difference was not statistically significant (one-way ANOVA, $p = 0.14$).

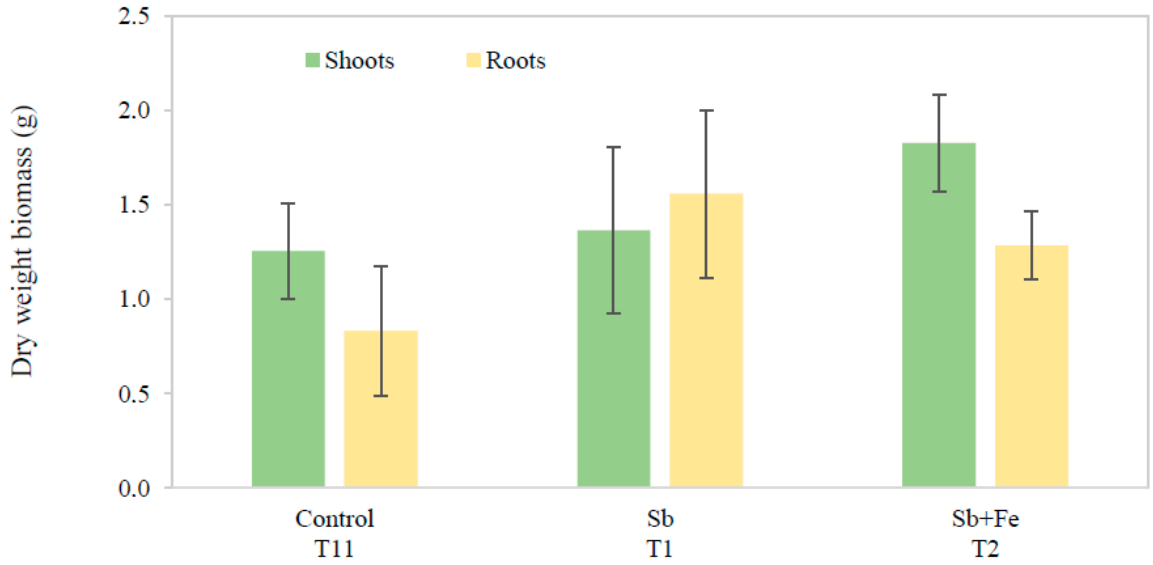


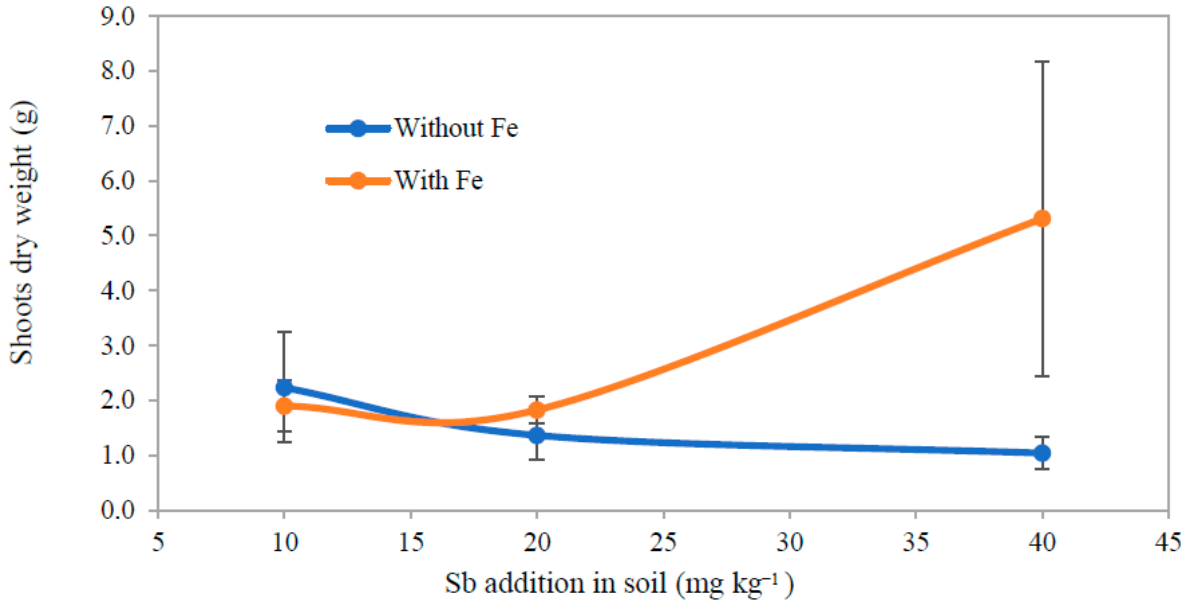
Figure 1. Effect of Sb and Sb+Fe addition on the produced biomass (t = 30 days).

3.1.2. Effect of Pollution Levels on Biomass Production

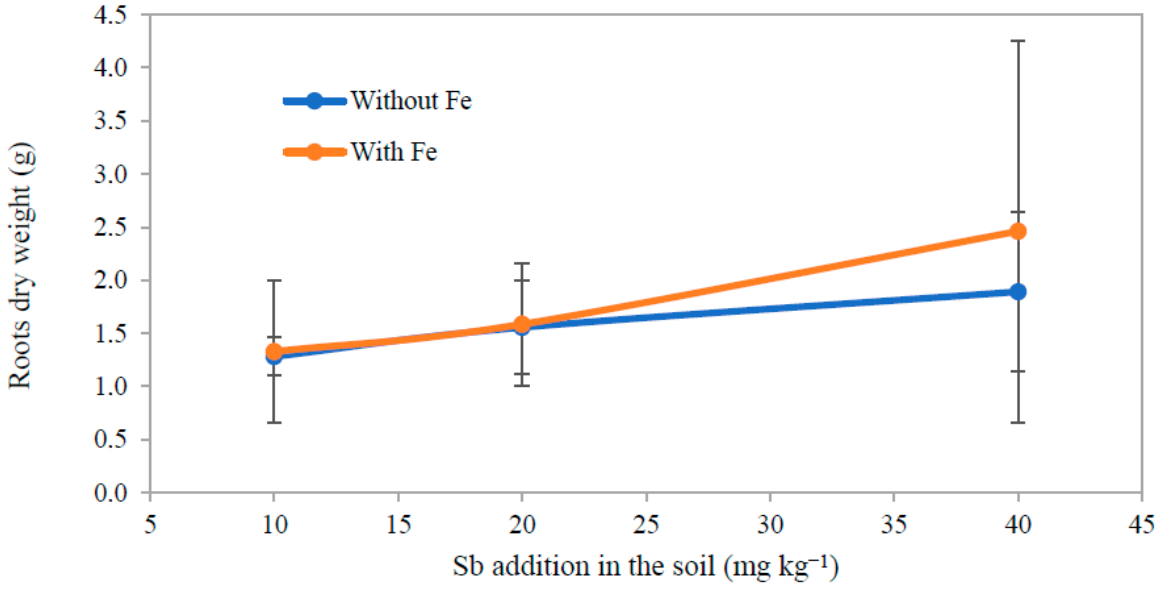
The effect of different levels of Sb contamination on the production of phytomass, without Fe and with simultaneous Fe addition, is presented in Figure 2. In the absence of Fe addition (Figure 2a), a small decrease in the weight of the plant mass was observed from 2.2 to 1.4 and 1.0 g, when the addition of Sb increased from 10 to 20 and 40 mg kg^{-1}. The differences are of course small and are assessed as not significant.

The addition of Fe at a molar ratio of Fe/Sb = 1.5 mole/mole, at the lowest pollution level (Sb = 10 mg kg^{-1}, Fe = 6.9 mg kg^{-1}), the aboveground plant mass production is similar to that without Fe, i.e., 1.9 g with Fe and 2.2 g without Fe. At the highest pollution level with the corresponding Fe addition (Sb = 40 mg kg^{-1}, Fe = 27.5 mg kg^{-1}) a large increase in aboveground plant mass production was observed. The average value was 5.3 g with Fe versus 1.0 g without Fe. Due to the large variation in the values in the repeated experiments with Fe at the highest level (test T6), the effect of Fe addition was evaluated as marginally not significant ($p = 0.06$).

The production of phytomass in the root system is presented in Figure 2b. As the addition of Sb and Sb+Fe increases, an increasing trend is observed in the average dry weight of the roots. However, the differences are not significant.



(**a**)



(**b**)

Figure 2. The effect of different Sb contamination levels on biomass production with and without Fe in (a) shoots and (b) roots.

3.1.3. Effect of Exposure Duration to Sb Contamination

Figure 3a presents the produced aerial phytomass as a function of exposure time to pollution without Fe and with addition of Fe. Without Fe addition, increasing the exposure time from 15 to 45 days did not affect the weight of aerial plant mass, which ranged from 1.2 to 1.4 g. In the experiments carried out with addition of Fe, the weight of shoots gradually increased from 1.1 g at 15 days, to 1.8 g at 30 days and 3.1 g at 45 days. The differences were assessed as statistically significant at $p = 0.03$. The positive effect of Fe on plant growth is consistent with the important role of iron in chlorophyll production and the entire photosynthesis process [30].

In the root system (Figure 3b), the weight of the produced biomass seems to stabilize after 30 days in the tests without Fe, while in the tests with Fe an increasing trend is discernible. However, the differences are characterized as statistically insignificant.

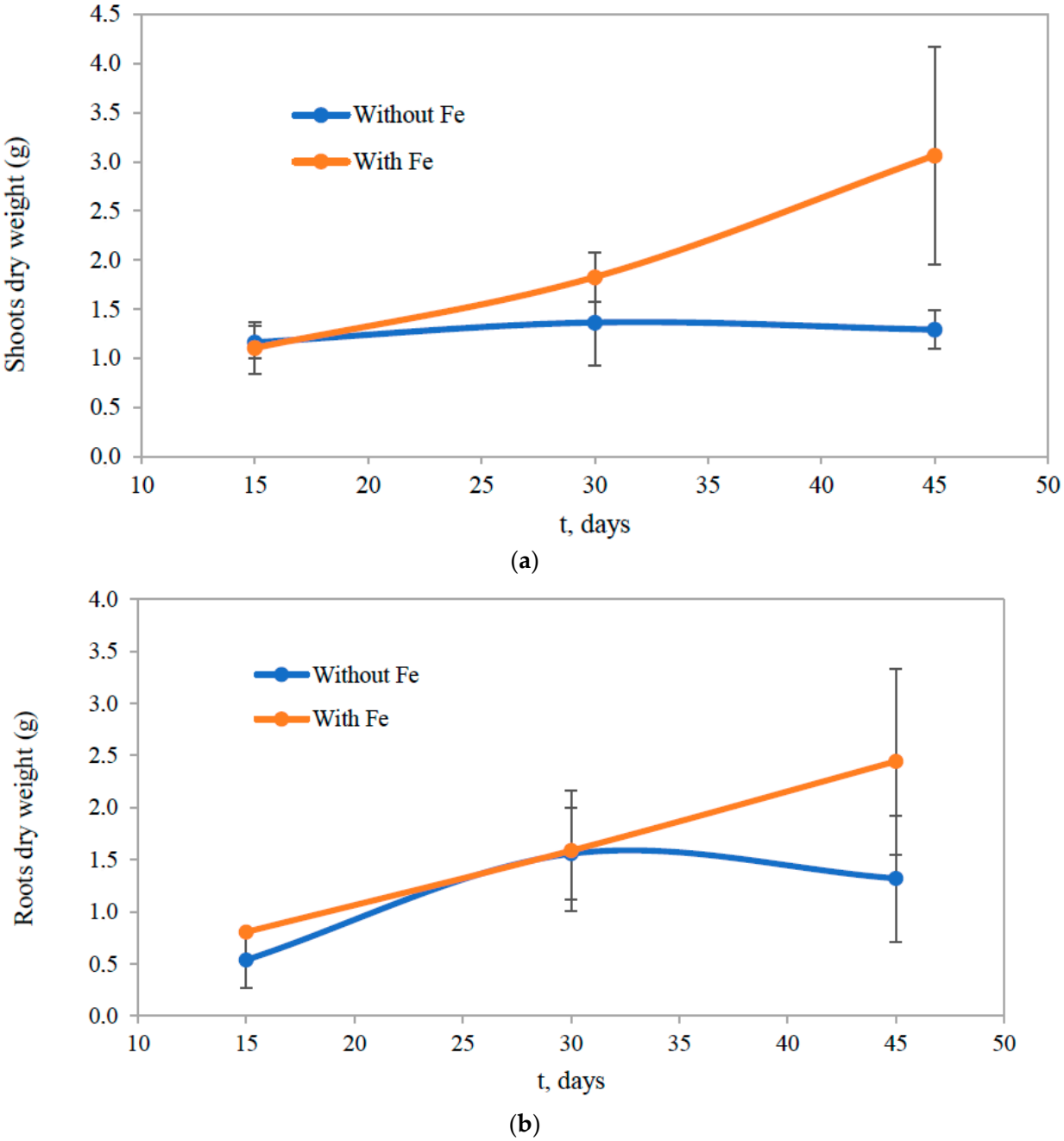


Figure 3. The effect of exposure duration to Sb contamination without Fe and with the addition of Fe on biomass production in (a) shoots and (b) roots.

3.2. Antimony Phytoaccumulation

3.2.1. Effect of Contamination Levels

Figure 4 presents the uptake of antimony (a) in the shoots (aerial parts) and (b) in the roots, as a function of the levels of soil pollution in Sb, without Fe and with the addition of Fe. Without the addition of Fe, the uptake in the shoots ranges from 2.6 to 3.3 mg kg^{-1}, without significant differences when the concentration of Sb in the soil increases from 10, to 20 and 40 mg kg^{-1} Sb. In the tests with the addition of Fe, very large variations were observed in the repeatability experiments, but a certain trend of increasing phytoextraction of Sb is evident. At the highest pollution level tested, Sb 40 mg kg^{-1} and Fe 27.5 mg kg^{-1}, the average Sb value in the aboveground plant mass was 8.3 mg kg^{-1}.

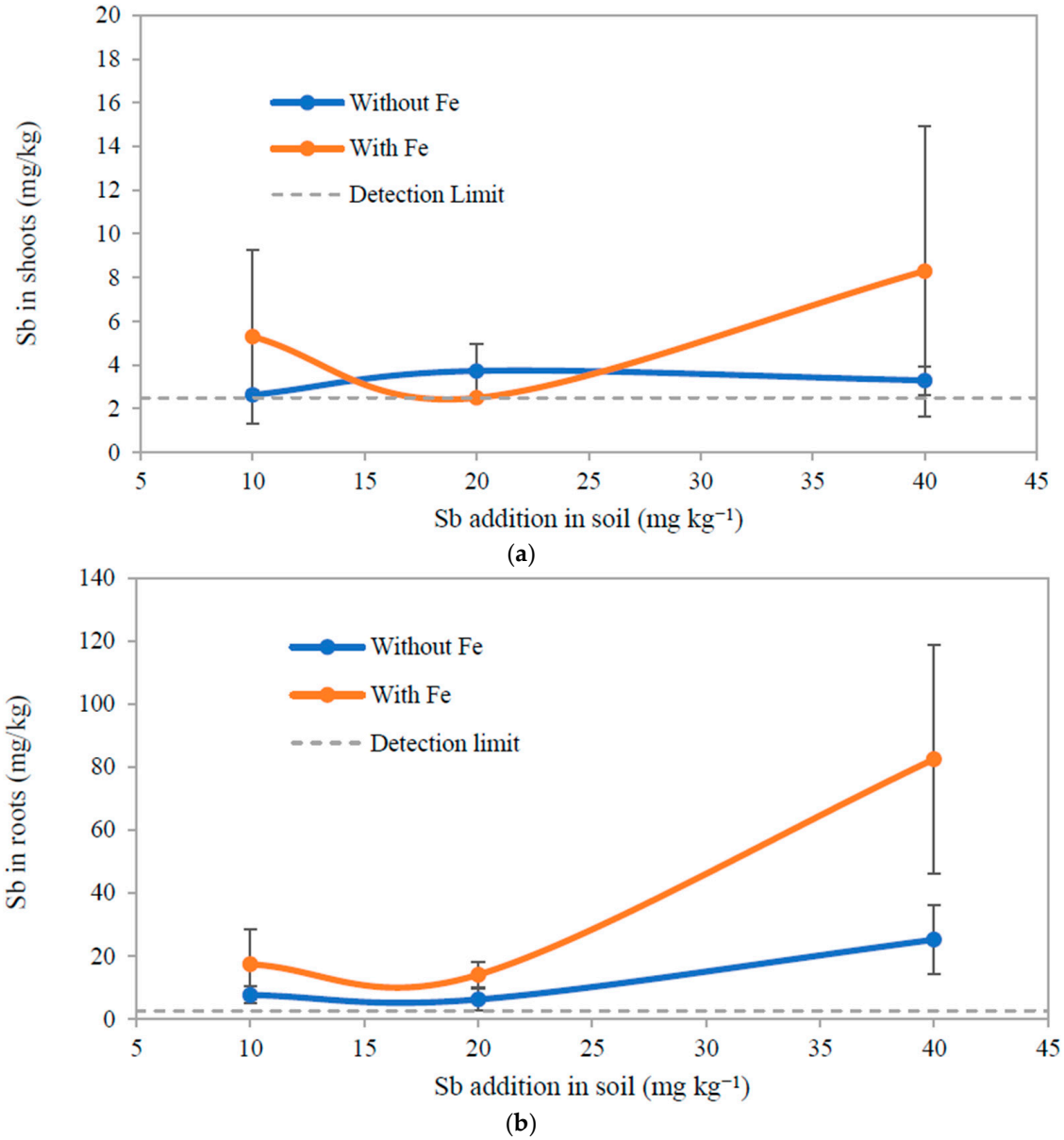


Figure 4. The effect of different Sb contamination levels on the phytoaccumulation of Sb, with and without Fe, in (a) shoots and (b) roots.

The levels of Sb uptake in the roots are much higher (Figure 4b). The concentration in the roots reaches up to 25 mg kg^{-1}, when only Sb (40 mg kg^{-1}) is present in the soil, and up to 82 mg kg^{-1}, when Sb (40 mg kg^{-1}) and Fe (27.5 mg kg^{-1}) are present simultaneously. The statistical evaluation of the results with the two-factor Anova method indicates that the combined factors, i.e., the increased levels of Sb contamination and the addition of Fe, have a statistically significant positive effect on the accumulation of Sb in roots.

3.2.2. Time-Dependent Sb Accumulation in Shoots and Roots

Antimony uptake in the aboveground parts and in the roots of plants as a function of exposure time to soil contamination is shown in Figure 5. Two treatments were examined: soil contaminated only with Sb at a concentration of 20 mg kg^{-1} and soil amended with Sb (20 mg kg^{-1}) and Fe (13.8 mg kg^{-1}). Increasing the exposure time from 15 to 30 and 45 days did not appear to affect Sb uptake in shoots, regardless of the presence or absence of Fe (Figure 5a). In contrast, Sb uptake in roots increased with increasing exposure time.

At 45 days, the mean Sb concentration in roots reached 40 mg kg^{-1} in the absence of Fe and 59 mg kg^{-1} in the presence of Fe (Figure 5b).

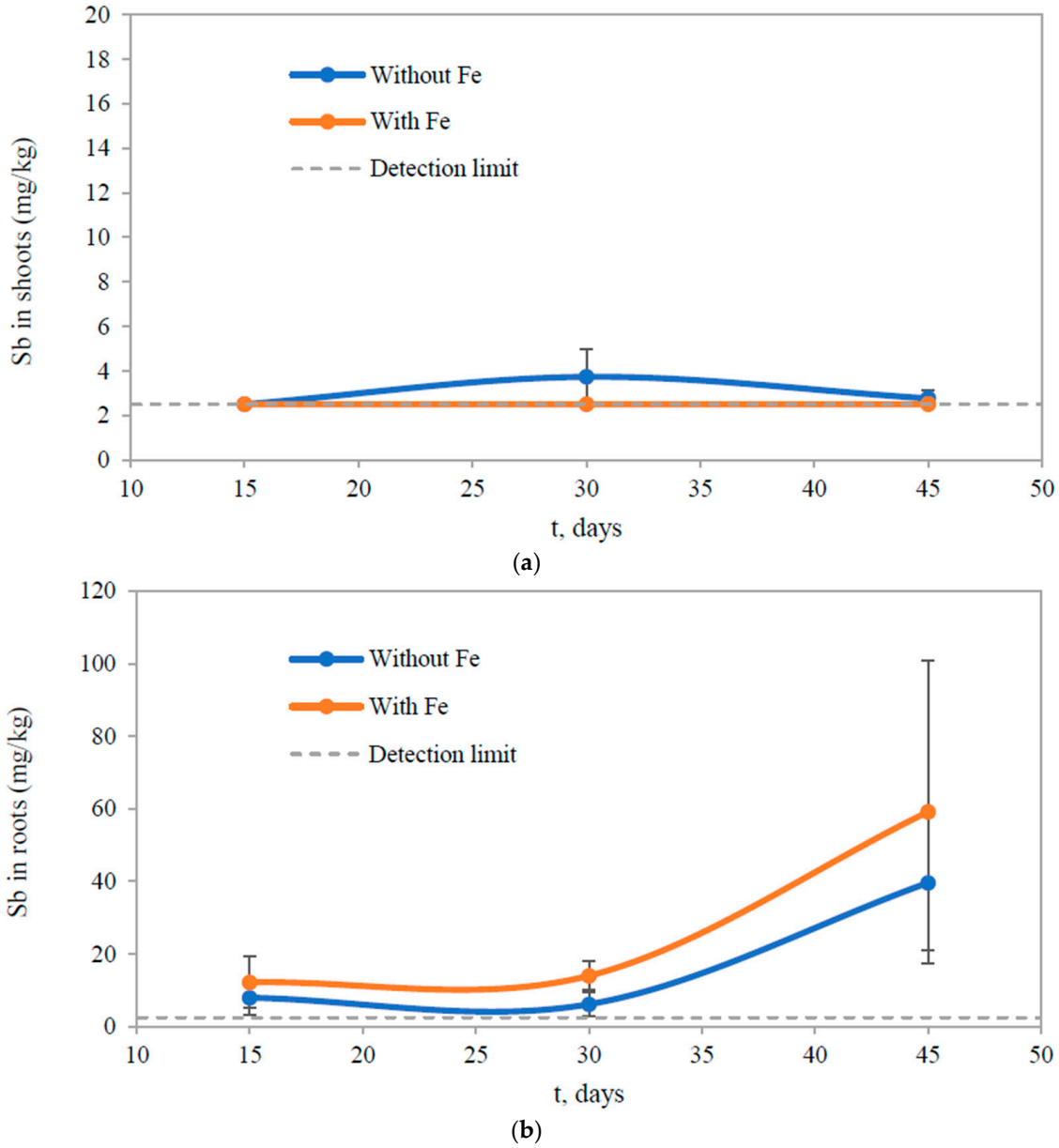


Figure 5. The effect of the duration of exposure to Sb contamination, without Fe and with the addition of Fe, on the phytoaccumulation of Sb in (a) shoots and (b) roots.

3.2.3. Translocation and Bioconcentration Factors

The translocation factor of Sb in the cardoon plants was found lower than 0.4, in all the examined conditions. The fact that antimony is mostly taken by plant roots has been observed by many researchers [2]. The uptake of Sb in roots is closely related to Sb speciation. In the pore water of soil Sb(V) occurs mainly as anion, $Sb(OH)_6^-$, while Sb(III) occurs as a neutral molecule, $Sb(OH)_3$. The neutrality of Sb(III) species facilitates its taken-up in the root system via the aquaporins of the cell membranes (apoplasmic pathway). Translocation to the aerial parts is limited by the Casparian strip, which blocks the passive flow of water and unwanted substances and allows the selective uptake of nutrients via the symplastic pathway. Contrary to Sb(III), the exact mechanisms of Sb(V) uptake in the roots and translocation to the aerial parts is not well understood [2].

Seridou et al. [31] studied the phytoaccumulation of Sb(III) in *Nerium oleander* and investigated the effect of adding several organic acids (citric, ascorbic, and oxalic acid) to stimulate the accumulation. Results revealed that Sb uptake in the roots was greater in the treatment with the highest organic acids concentration, with a bioconcentration factor greater than 1.0. However, the translocation of Sb for every treatment was very low, confirming that *N. oleander* plant cannot transfer Sb from the root to the shoots.

The aerial BCF values recorded in this study ranged from 0.08 to 0.53, suggesting limited potential for phytoextraction. In contrast, the root BCF values ranged from 0.31 to 2.96, indicating some capacity for phytostabilization, particularly with the addition of Fe (see Figure 6).

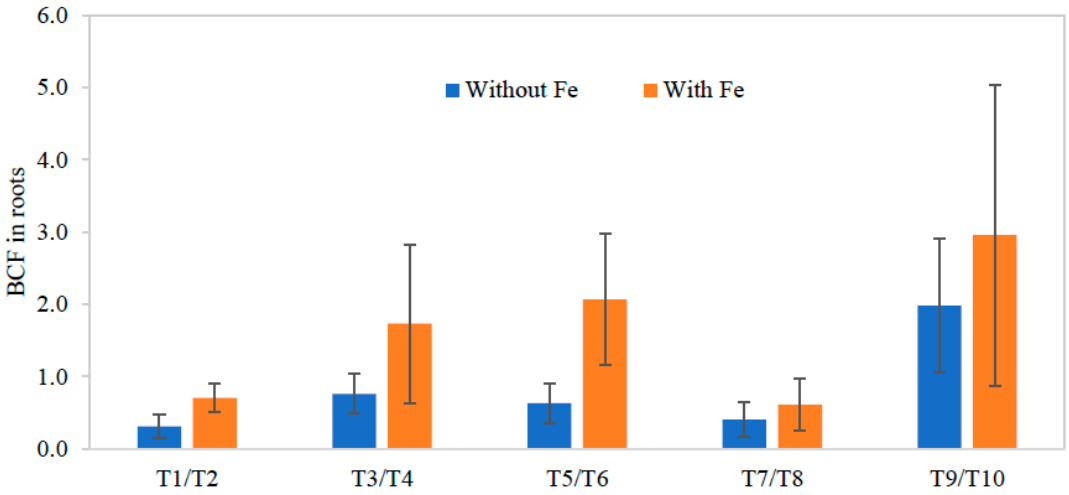


Figure 6. The bioconcentration factors BCF in the roots of C. cardunculus, without Fe and with the addition of Fe.

The total phytoaccumulation of Sb (in shoots and in roots) versus the amount of Sb added in the soil was very low (see Figure S1 in Supplementary Material). Namely, the highest phytoaccumulation was equal to 0.77% with respect to Sb addition in soil and was recorded in test T10 (added Sb = 20 mg kg^{-1}, Fe(II) 13.8 mg kg^{-1} and total duration 45 days).

It is also noted that the phytoaccumulation results for the cardoon plant cultivated in the initial soil (test T11) showed that the antimony was below detection limit both in the shoots and in the roots of the plants in all the replicate pots. This indicates that geogenic Sb (12.7 mg kg^{-1}) is not bioavailable, in agreement with the undetectable water solubility according to the EN12457-2:2002 test [29] (water soluble Sb < 0.05 mg kg^{-1}, Table 2).

3.3. Water Soluble Antimony

3.3.1. Comparison of Tests with and Without Plants

Figure 7 illustrates the effect of *Cynara cardunculus* planting and Fe addition on the water-soluble fraction of antimony. Water soluble Sb was determined using the standard leaching test EN12457-2:2002 [29]. The experiments were conducted with a total Sb concentration in soil 20 mg kg^{-1}. The water-soluble fraction was determined 30 days after the application of artificial contamination. In test T12 which involved Sb addition only (20 mg kg^{-1}) and no plant, the water-soluble Sb fraction after 30 days was 2.1 mg kg^{-1}, indicating an approximately five-fold reduction due solely to the soil itself. This observation suggests the occurrence of natural attenuation. Natural attenuation is a known process of reducing the solubility-mobility of metals and metalloids in soils, through various geochemical processes, mainly adsorption on iron, aluminum and manganese oxides, precipitation

in the form of solid compounds, etc. [32]. Addition of Fe (T13) further enhanced Sb stabilization, reducing the water-soluble fraction to 1.5 mg kg^{-1}. This additional decrease in solubility may be attributed to the precipitation of the solid phase schafarzikite ($FeSb_2O_4$), which forms through interactions between trivalent antimony, Sb(III), and divalent iron, Fe(II) [3].

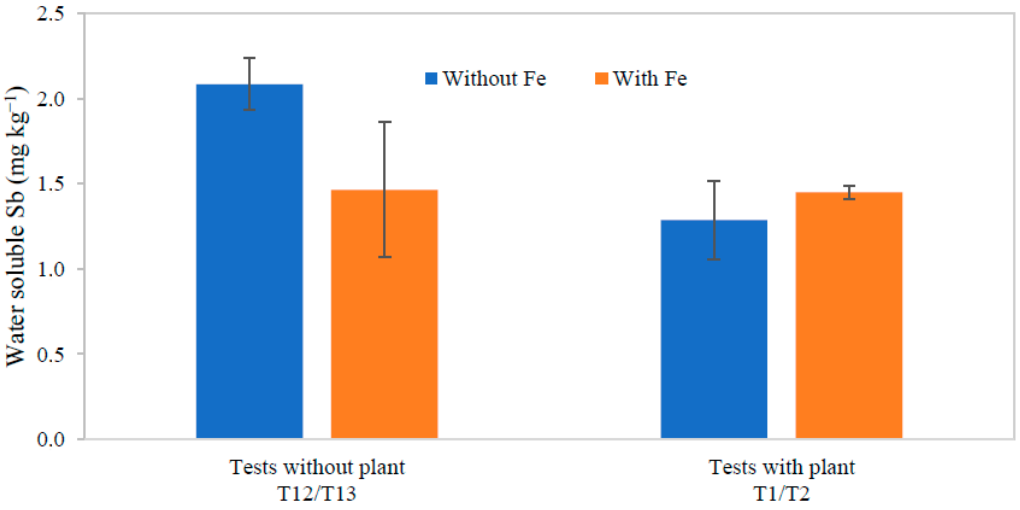


Figure 7. Effect of *C. cardunculus* planting and addition of iron to the water-soluble antimony (total Sb =20 mg kg^{-1}, t = 30 days).

The presence of wild cardoon (*C. cardunculus*) in the contaminated soil exhibited a clear stabilizing effect in the absence of Fe addition (significance level p = 0.01). In the treatment involving Sb and *Cynara cardunculus* only (test T1), Sb solubility decreased by 38% compared to control treatment (T12).

3.3.2. Effect of Total Antimony Levels

The concentration of water-soluble antimony in soil as a function of total antimony contamination levels, with and without addition of Fe, is presented in Figure 8. The experiments were all carried out with plants, and the soil samples for the determination of water-soluble Sb were taken 30 days after the application of artificial contamination.

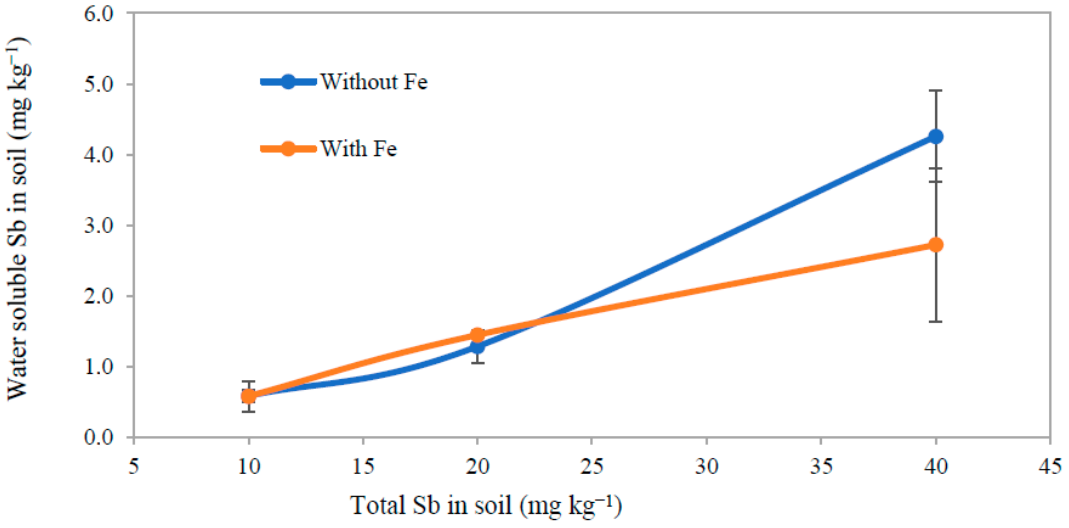


Figure 8. The water-soluble Sb in soil, as a function of total Sb contamination levels with and without Fe addition (experiments with plants, t = 30 days).

At the lower Sb contamination levels, 10 and 20 mg kg^{-1}, the water-soluble Sb was similar in the experiments carried out with or without addition of Fe. At the highest total Sb concentration (40 mg kg^{-1}), iron addition appeared to enhance Sb stabilization, reducing the water-soluble Sb concentration from 4.3 to 2.7 mg kg^{-1}. The difference, however, was not assessed as statistically significant (p = 0.16).

3.3.3. Temporal Evolution of Water-Soluble Antimony

The temporal evolution of water-soluble Sb with and without addition of iron is shown in Figure 9. In these experiments, the total Sb concentration was kept constant at 20 mg kg^{-1}. As seen in Figure 9, cultivation of cardoon, without addition of Fe, caused an almost linear decrease in the water-soluble antimony from 1.75 to 0.86 mg kg^{-1} as the time increased from 15 to 45 days. In this set of experiments, the effect of time was evaluated as statistically significant (p = 0.009). When the cultivation of cardoon was combined with the addition of Fe, the recorded mean values of the water-soluble Sb were 1.53, 1.45 and 1.16 mg kg^{-1} after 15, 30 and 45 days, respectively. Taking into consideration the variances among replicate experiments, this slight decrease in the mean values was evaluated as insignificant.

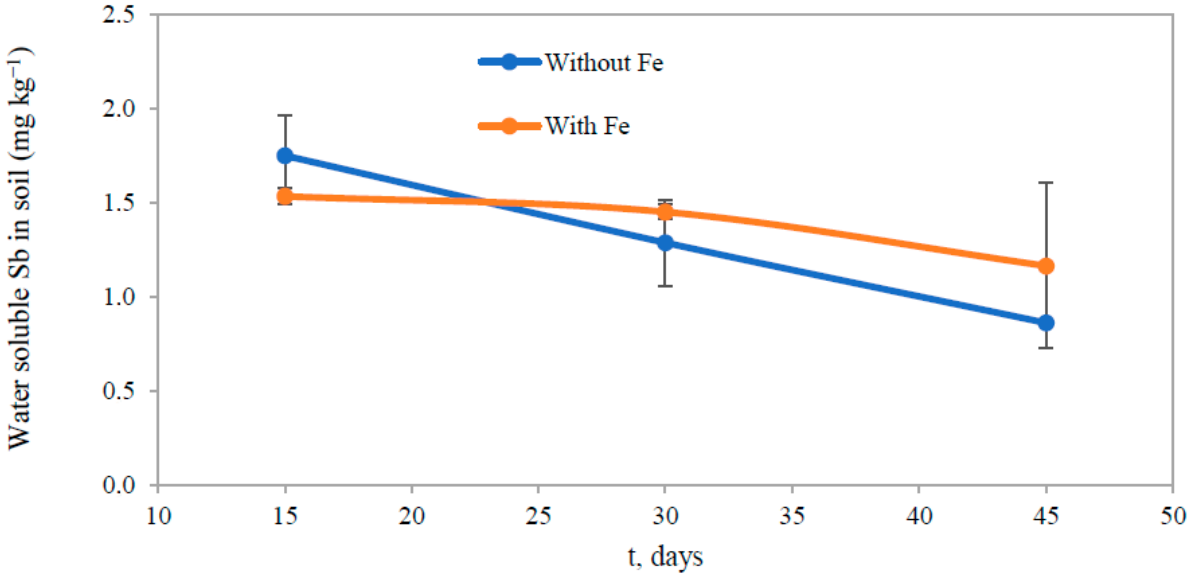


Figure 9. The temporal evolution of water-soluble Sb in soil with and without Fe addition (experiments with plants, Sb total = 20 mg kg^{-1}).

At this contamination level and within the time scale examined, the stabilizing effect of *Cynara cardunculus* appears to increase with time, without requiring Fe addition.

4. Discussion

This study was carried out using potassium antimony tartrate as Sb(III) source for the artificial contamination of soils. The tartrate salt was selected, because it is a highly water-soluble compound that has been widely employed to prepare dissolved Sb(III) solutions under controlled laboratory conditions. Relevant thermodynamic and spectroscopic studies have demonstrated that Sb(III) forms stable complexes with low-molecular-weight organic ligands, including tartrate, through hydroxyl and carboxyl functional groups [33,34]. Consequently, tartrate complexation may transiently influence the initial solubility, adsorption behaviour, and mobility of Sb(III) following its introduction into the soil. However, under soil conditions, Sb(III) readily undergoes hydrolysis and its environmental behaviour is predominantly controlled by interactions with soil constituents, particularly Fe oxides and

hydroxides, clay minerals, and soil organic matter, which govern its adsorption, speciation, and bioavailability [17]. Since all Sb-amended treatments received the same Sb(III) source and were subjected to identical experimental conditions, any potential influence of the tartrate ligand was common to all treatments and therefore does not affect the comparative evaluation of Fe amendment or the phytoremediation performance of *Cynara cardunculus*. Nevertheless, the possible influence of tartrate complexation on the initial behavior of Sb(III) should be considered when comparing the present findings with studies employing different Sb(III) salts.

In the present work relatively low contamination levels of antimony (10–40 mg kg^{-1}) were investigated, reflecting the low geochemical background of Sb in soils, particularly in Greece, where the median Sb concentration is approximately 0.84 mg kg^{-1}. Within this concentration range, no pronounced phytotoxic effects were observed, as evidenced by the absence of significant reductions in aboveground biomass production. Biomass yields in Sb-amended soils (1.0–2.2 g) were comparable to those of the uncontaminated control (1.3 g), indicating a high tolerance of *Cynara cardunculus* to Sb stress at environmentally relevant contamination levels.

Iron addition had a decisive positive effect on plant growth. At the highest Sb concentration (40 mg kg^{-1}), the simultaneous addition of Fe at a molar Fe/Sb ratio of 1.5 resulted in a fivefold increase in biomass production compared to treatments without Fe. Moreover, Fe supplementation promoted sustained biomass accumulation over time. At 20 mg kg^{-1} Sb, biomass production remained nearly constant without Fe between 15 and 45 days, whereas Fe-amended treatments exhibited more than a twofold increase during the same period.

The beneficial effect of Fe addition on cardoon growth is likely attributable to multiple interacting mechanisms, including improved Fe nutrition under alkaline soil conditions, mitigation of oxidative stress, and indirect rhizosphere-mediated processes, rather than solely to the immobilization of Sb.

The experimental soil was alkaline (pH 8.2), a condition that markedly reduces Fe bioavailability by promoting the rapid oxidation of Fe(II) to Fe(III) and the precipitation of poorly soluble Fe(III) hydroxides. Consequently, plants grown in calcareous soils frequently experience iron deficiency, which impairs chlorophyll biosynthesis and photosynthetic activity. The addition of Fe(II) likely increased the pool of soluble Fe during the early stages of cultivation, thereby enhancing chlorophyll synthesis and photosynthetic performance. This mechanism may partly explain the greater biomass production observed in Fe-amended treatments.

On the other hand, antimony toxicity is known to induce oxidative stress through excessive production of reactive oxygen species (ROS), resulting in lipid peroxidation, membrane damage and inhibition of plant growth. Iron is an essential micronutrient involved in numerous redox enzymes and photosynthetic proteins and may contribute to maintaining cellular redox homeostasis when supplied at appropriate concentrations. Several studies have reported that Fe supplementation enhances the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and reduces malondialdehyde (MDA) accumulation thereby alleviating oxidative damage and improving plant tolerance to metalloid stress [35,36]. Although these physiological parameters were not determined in the present study, this mechanism may partly explain the improved growth of *Cynara cardunculus* following Fe amendment.

Despite the favorable growth response, antimony uptake into aboveground tissues (stems and leaves) was very limited. The calculated bioconcentration factor (*BCF*) ranged between 0.08 and 0.53, values far below those required for effective phytoextraction. In phytoextraction projects, the total time required to reduce the pollutant concentration in the

soil from an initial value C_{s0} to the final desired value C_{sf} can be approximately calculated from Equation (1)

$$t = ln\left(\frac{C_{s0}}{C_{sf}}\right)\frac{10000\, h_s d_s}{\Upsilon \cdot BCF} \quad (1)$$

where t is the required time in years (y), h_s is the depth of contamination (m), ds the bulk density of soil (kg m^{-3}), Υ the yield of dry biomass per hectare and year (kg ha^{-1} y^{-1}) and BCF the bioconcentration factor. *C. cardunculus* is a plant with high productivity, in the order of 15 t ha^{-1} y^{-1} [37]. Assuming depth hs = 0.3 m, soil density ds= 1200 kg m^{-3} and BCF = 0.5, it is calculated that the time required to reduce soil Sb concentrations from 20 to 5 mg kg^{-1}, would be 665 years. Consequently, *C. cardunculus* does not present a realistic option for antimony phytoextraction.

In contrast, the results clearly demonstrate a strong phytostabilization effect. Cultivation of *C. cardunculus* alone led to a 38% reduction in the water-soluble fraction of Sb in soil, even in the absence of Fe addition. Furthermore, this stabilizing effect increased over time, with water-soluble Sb decreasing from 1.75 mg kg^{-1} at 15 days to 0.86 mg kg^{-1} at 45 days. The reduction in bioavailable Sb suggests immobilization processes occurring in the rhizosphere, potentially driven by root-induced changes in soil chemistry, adsorption onto root surfaces, or complexation with soil constituents enhanced by plant activity.

Cardoon develops an extensive root system capable of releasing organic acids, phenolic compounds and other root exudates that modify rhizosphere chemistry and influence metal(loid) mobility. Furthermore, *Cynara cardunculus* rhizosphere is a dynamic microbial environment rich in beneficial bacteria and fungi. These microorganisms, notably Arbuscular Mycorrhizal Fungi (AMF) are known to contribute to the immobilization of heavy metals and metalloids, including antimony [38,39].

The dominant phytostabilization behavior of *C. cardunculus* is particularly advantageous for Sb-contaminated sites, where preventing leaching and ecological dispersion of the metalloid is often more critical than its complete removal. By reducing Sb mobility and bioavailability, the plant limits risks to groundwater and food chains while maintaining soil structure and vegetation cover [40]. Nevertheless, several limitations must be considered. Phytostabilization does not remove antimony from the site but rather immobilizes it, requiring long-term land use control and monitoring. Additionally, the effectiveness of stabilization may depend on soil properties, Sb speciation, and climatic conditions [41–43]. Lacalle et al. [44] evaluated the phytostabilization in two types of contaminated soil (agricultural and mining) with Cd, Pb, Zn and Cu using combination of *B. juncea* and *C. cardunculus* in a field scale application. The combination of *B. juncea* and *C. cardunculus* decreased phytotoxicity and soil microbial communities were slightly stimulated. Previous research has also demonstrated that the addition of organic amendments is often necessary in nutrient-deficient or heavily contaminated soils to support plant establishment and early growth [44–46]. From an applied perspective, the combination of Sb phytostabilization with biomass production presents a promising opportunity. The substantial increase in biomass following Fe addition is particularly relevant for integrating phytoremediation with bioenergy production, potentially improving the economic viability of remediation efforts. However, careful management of harvested biomass is essential to avoid secondary contamination risks. Overall, *Cynara cardunculus* exhibited high tolerance to antimony-contaminated soils and a pronounced phytostabilizing effect, which intensified over time. These characteristics, together with its adaptability to Mediterranean environments and high biomass potential, support its suitability for long-term, low-input phytostabilization strategies at Sb-contaminated sites. Future research should focus on field-scale validation,

long-term stability of immobilized Sb, and optimization of soil amendments to further enhance stabilization efficiency.

5. Conclusions

This study demonstrates that *Cynara cardunculus* is a promising candidate for the phytostabilization of Sb-contaminated soils. Under the investigated conditions, the species exhibited high tolerance to Sb contamination, maintaining normal growth even at Sb concentrations up to 40 mg kg^{-1}. Antimony accumulation occurred predominantly in the root system, while translocation to the aerial biomass remained limited (TF < 0.4), indicating that the plant is more suitable for phytostabilization than for phytoextraction. Moreover, the presence of cardoon reduced the water-soluble fraction of Sb in soil by up to 50%, confirming its ability to decrease Sb mobility and bioavailability. These findings represent the first evidence that *C. cardunculus* can be successfully applied for the phytostabilization of Sb-contaminated soils.

The addition of Fe(II) further improved the performance of the system. Although Fe supplementation had only a moderate effect on Sb stabilization, it significantly enhanced plant growth, particularly at longer exposure times, increasing aboveground biomass by up to 2.3-fold. Furthermore, Fe promoted Sb retention within the root system, resulting in higher root bioconcentration factors while maintaining low translocation to the shoots. These observations suggest that Fe(II) may contribute to improved plant tolerance to Sb stress while simultaneously supporting phytostabilization processes.

From a practical perspective, the use of *C. cardunculus* offers a dual environmental benefit. As a perennial, high-biomass bioenergy crop adapted to Mediterranean environments and marginal lands, it provides the opportunity to combine the ecological restoration of contaminated sites with biomass production for renewable energy, thereby improving the economic sustainability of phytomanagement strategies. This approach may be particularly attractive for mining-affected areas where conventional remediation techniques are often economically prohibitive.

Future research should focus on long-term field-scale validation of the proposed remediation strategy, investigation of the physiological mechanisms underlying Fe-enhanced Sb tolerance, including chlorophyll synthesis, oxidative stress responses and rhizosphere interactions, and evaluation of Sb speciation and bioavailability under natural environmental conditions. In addition, the agronomic performance and bioenergy potential of *C. cardunculus* should be assessed under field conditions to further establish its suitability as a sustainable phytomanagement crop.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environremediat1020008/s1.

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