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Applicability of a Freundlich-Like Model for Plant Uptake at an Industrial Contaminated Site with a High Variable Arsenic Concentration

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Abstract: Phytoextraction is a low-cost technology with negligible environmental impacts. A major issue at the field scale is the heterogeneity of contaminant concentration since the entire site needs to be treated evenly even though zones may need different incisiveness in the treatment. The concentration ratio (C_{shoot}/C_{soil}) is generally used to evaluate plant species performance and it includes for simplicity an assumption of linearity in the uptake behavior, although deviation from linearity has been observed in several studies. This work describes a phytoextraction feasibility test, conducted at a greenhouse scale for the remediation of an arsenic-contaminated site. Since a feasibility test should also provide an uptake model that accounts for plant growth in heterogeneous areas, the investigation focused on defining the uptake behavior of the various selected species growing in a site with homogeneous soil properties, but with considerable differences in arsenic concentration. Among the many models selectable to describe the soil-to-plant transfer, the Freundlich-like approach was tested. While remaining easy to handle, the non-linear model selected proves to be adequate to predict the arsenic uptake despite the complex contamination considered, thus allowing a more realistic prediction of the potential of a field-scale phytoremediation procedure.

Keywords: arsenic; assisted phytoextraction; bioavailability; contaminated soil; Freundlich-like model; modeling uptake; phytoremediation

1. Introduction

The presence of heavy metals in contaminated soils is of great concern as they are not biodegradable and thus pose a risk for humans and the environment. Among them, arsenic (As) is one of the most common metals in contaminated sites because of its widespread accumulation in the soils of former industrial sites [1].

Different approaches have been developed to recover a site affected by inorganic contamination, but most of the conventional technologies (e.g., landfilling or soil washing) are nowadays no longer considered sustainable compared with emerging innovative approaches [2]. Indeed there is an increasing interest in green technologies aimed not only at eliminating contamination from soil but also at protecting the long-term environmental sustainability of soil functions, thus conserving its quality [3]. The key aspects of the increasing success of these green remediation technologies lie in the negligible environmental impacts and low costs. Among these green technologies, phytoremediation is considered of great interest since it employs the plants for the in situ treatment of contaminated soil and water. In addition to remediating the compromised area, phytoremediation also restores the soil quality with minimal disturbance of the surroundings of the contaminated site.

Phytoextraction is an active phytoremediation method that exploits the plants' ability to take up, accumulate and concentrate metals from the soil in their harvestable tissues. The soil-to-plant transfer of heavy metals leads to decontamination. The efficiency of phytoextraction is strongly related to the plant species and to the bioavailability of contaminants in the soil. Plants that are able to grow in soils with high metal concentrations (hyperaccumulators) and/or with high biomass crop production are suitable for phytoextraction. The metal bioavailability, namely the mobile and available fraction in soil for uptake by plants, is largely conditioned by the soil properties, which determine the distribution of the contaminants between the solid and liquid soil phases. Generally, the pH, clay and organic matter contents in soil regulate the uptake of metals by plants due to adsorption-desorption mechanisms [4]. The toxicity and bioavailability of arsenic are also affected by biogeochemical factors and greatly depend on the oxidation state and chemical species, which are controlled by the redox conditions [5]. For example, arsenite (AsIII) is more available in anaerobic soil and is more mobile and toxic than arsenate (AsV), which, on the contrary, is largely the prevalent form in well-aerated soils [6]. Arsenic is also involved in complexation reactions on the oxide and hydroxide surfaces of Fe, Al, Mn, especially in the clay size fraction of soil [7].

One of the problems of phytoremediation at the field scale is the heterogeneity of contamination at the same contaminated site. A split phytoremediation treatment in small plots is not possible, but the entire contaminated site needs to be treated evenly, despite the different levels of contamination in different areas. In cases like the site discussed in this paper, with a high variability of As concentrations, this heterogeneity obviously needs to be taken into account when estimating the contamination baseline to be used when designing the remediation process [8].

Feasibility tests should therefore be based not only on experimental data on plant growth and the accumulation of contaminants in plant tissues, but should also provide an uptake model that accounts for plant growth in areas with different concentrations at the same contaminated site. The purpose of modeling is to predict the transfer of contaminants from soil-to-plants.

The evaluation of phytoextraction efficiency using a feasibility test scale is generally based on the ratio of concentration of contaminants in the shoots and the total concentration in soil ($CR = C_{shoot}/C_{soil}$). The concentration ratio, also called the phytoextraction coefficient (PEC), can be used to assess the effectiveness of plants in removing metals from the soil [9] in a short-growing period. However, in phytoextraction, only the metal "bioavailable fraction" is the amount that can be taken up by plants [10]. Thus, for phytoextraction purposes, it is also useful to calculate the bioavailability factor (BF), i.e., the ratio between the metal concentration in shoots and the potential bioavailable concentration in the soil. The translocation factor (TF) defines the ratio of metal concentration between the aboveground and belowground biomass and provides an estimate of the translocation of metals within the plant [11]. PEC (or BF) and TF results can be used to assess the plant's ability to take up metals from the soil and to translocate them to harvestable tissues, and provide a (only) preliminary evaluation of the potential efficiency of phytoremediation.

These indexes also allow distinguishing between hyperaccumulator species (PEC and TF ≥ 1) [5] and metal tolerant plants [11], where PEC or BF values greater than 0.5 still satisfy the criteria for phytoextraction applicability [9,12], due to a greater biomass production and a rapid growth rate, thus a higher total extraction of metals than hyperaccumulators [13,14].

In general, the use of indices such as CR for evaluation of treatment entails an assumption of linearity: the concentration in plants increases with an increasing soil concentration as described by the CR ratio specific for each contaminant and each plant. However, deviation from linearity has been observed in many contaminant uptake studies [15,16]: plants generally take up elements more efficiently at low soil concentrations compared to high soil concentrations, thus deviating from linearity with a decrease in CRs with increasing soil concentrations. The use of non-linear functions to take into account this behavior has been proposed by several authors [17,18].

In recent years several models of plant uptake have been put forward. Very complex models that describe the behavior of organic [19,20] and inorganic [21,22] contaminants in plants have been developed. The aims of these models vary, for example, to determine the risk to human health from contaminated plants, to address theoretical aspects of phytoremediation, and to assess the interactions between the biogeochemical cycles of the soil and atmospheric impacts.

For practical use in real-scale, reclamation is preferred by far a very simple model, which can be applied immediately to provide the essential data to plan a field test and this is why it usually ends up adopting a linear one. However, such an approach often leads to an inevitable overestimation of the efficiency of the treatment, a factor that, especially in conditions of complex contamination, adds further uncertainty to a design already difficult, leading to high risk of not achieving the desired specifications. On the contrary, there are non-linear models, such as the Freundlich-like one, which at the cost of a slightly higher complexity might yield much more reliable results.

This work reports the data of a phytoextraction feasibility test, conducted at a greenhouse scale for the reclamation of a site contaminated by a highly variable arsenic concentration. Three plant species were compared and the effectiveness of the addition of phosphate to increase As bioavailability was verified.

The soil-to-plant transfer of As was studied to verify the applicability of a Freundlich-like approach in order to obtain a simple but more reliable prediction equation to define the uptake behavior of different plant species in soil with homogeneous physical chemical properties, and similar fertility parameters (NPK) but also characterized by adjacent areas presenting a considerable variability in As concentration.

2. Materials and Methods

2.1. Soil Sampling and Characterization

The soil used in this study was collected from an industrial site located in Tuscany, Italy. The site had been used for various industrial productions and contamination from various inorganic contaminants has been detected. Arsenic was among the major pollutants found, with a considerable heterogeneity in concentration in the area under examination. The site was subdivided according to different Thiessen polygons. Soil samples for a phytoextraction feasibility test were collected from the five most representative polygons, named A, B, C, D, and E, in increasing order of As total concentration. The soil samples were air dried and sieved with a 2 mm sieve, and then homogenized and analyzed.

The physical and chemical properties of the soils were determined according to standard methods [23]: pH, using a glass electrode in a 1:2.5 soil/water ratio, cation exchange capacity (CEC) using barium acetate, texture (sand, silt, and clay) via the pipette method and organic matter (OM) content by RC-412 Multiphase Carbon. The total concentration of As was determined via acid digestion with an HNO_3 (65%, v/v) and H_2O_2 (30%, v/v) mixture in a PTEF-TMF (polytetrafluoroethylene-tetra-fluoromethoxil) pressure digestion vessel using a microwave oven (FKV-ETHOS 900), in accordance with the EPA method 3051-A [24].

The potential bioavailable As was evaluated by the first two steps of modified Wenzel's sequential extraction [25] adding 0.05 M $(NH_4)_2SO_4$ (ammonium sulfate) and 0.05 M KH_2PO_4 (potassium dihydrogen phosphate), sequentially.

The extraction was performed by shaking the soil and extractant (ratio of 1:25) for 2 h, using 50 mL polypropylene centrifuge tubes. Each extraction was run in triplicate and the As content was analyzed in the extracts after centrifugation at 15,000 rpm for 15 min, and after filtration.

2.2. Mesocosm Experiment

The phytoextraction experiment was conducted at the mesocosm scale in a greenhouse, where the temperature and humidity were 18–25 °C and about 65%, respectively, with natural day/night cycles. Further details are reported in a previous work [26].

Three plant species were selected for the experimental trials, *Brassica juncea* var. Scala, *Lupinus albus* var. Multitalia, and *Helianthus annuus* var. Paola, all with a positive metal tolerance and accumulation potential [27–29].

In order to obtain a representative sample of the field situation, mesocosms (i.e., polypropylene containers) were filled with 5 kg of soil, from which the coarser materials had already been eliminated (sieving at 2 cm). A hole in the middle of the base of each pot, connected with a PVC tube to a plastic bottle, enabled the leachates to be collected easily [30].

A total of 0.5 g per pot of *B. juncea* seeds, 12 and 9 seeds per pot of *L. albus* and *H. annuus*, respectively, were sown. Three replicates for each species were treated with K_2HPO_4 (T) as As mobilizing agent, with controls (CT) running simultaneously. During the growing period, plants were watered daily according to the plants' needs without additional nutrients.

In order to prevent or at least to minimize possible toxic effects on plant species, the treatments with 0.1 M K_2HPO_4 were started about thirty days after sowing, by splitting the total dose into consecutive five-day applications [31], each with 50 mL per pots.

The whole experiment lasted 60 days. At plant harvest, the aerial parts were separated from the roots and all samples were washed with deionized water. The roots were further washed in an ultrasound bath (Branson Sonifier 250 ultrasonic processor, Branson Ultrasonics Corporation, USA) for 10 min to eliminate any soil particles remaining on root surfaces. The dry biomass of vegetal samples was then gravimetrically determined after the samples had been dried in a ventilated oven at 40 °C until a constant weight was achieved. The dry plant samples were ground, homogenized, and digested with an acid mixture (HNO_3 + H_2O_2) for As determination, according to EPA method 3052 [32]. Before and after the addition of phosphate, leachate samples from all pots were also collected.

2.3. Arsenic Quantification and Quality Control

Arsenic concentrations in digested samples and in soil extracts were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) with a Liberty AX Varian spectrometer. Arsenic content in leachates was also quantified via ICP-OES after filtration at 0.6 µ. Metal concentrations were expressed in milligrams per kilogram dry weight ($mg \cdot kg^{-1}$) for soil and vegetal samples, or micrograms per liter ($\mu g \cdot L^{-1}$) for leachates. All data reported are the average of three replicates.

Quality assurance and quality control were performed by testing the two standard solutions (0.5 and 2 $mg \cdot L^{-1}$) every 10 samples. Certified reference materials (BCR n°141) were used to control the quality of the analytical system. The detection limit was 50 $\mu g \cdot L^{-1}$ for As. The recovery of spiked samples (5%) ranged from 93% to 101% with a relative standard deviation (RSD) of 1.91 of the mean.

2.4. Statistical Analysis

Statistical analysis was performed using STATISTICA version 6.0 (Statsoft, Inc., Tulsa, OK, USA). Treatment effects were analyzed using one-way analysis of variance (ANOVA). Differences among means were compared and a post-hoc analysis of variance was performed using the Tukey Honestly Significant Difference test ($p < 0.05$).

3. Results and Discussion

3.1. Soil Analysis

The physical and chemical properties of the soil were found to be homogeneous in the whole study area and were as follows: pH 8.2, CEC 17.5 $cmol_{(+)} \cdot kg^{-1}$, clay 8.4%, silt 18%, sand 73.6%

and OM 3.53%, N 0.15%, exchangeable K 185 mg·kg^{-1}, available P 2.5 mg·kg^{-1}. Conversely, total concentrations of arsenic ranged from about 25 (soil sample A) to 2500 (soil sample E) mg·kg^{-1} in the selected sampling points.

In all sampling areas, except polygon A, the As concentrations exceeded the threshold limit value (50 mg·kg^{-1}) required by Italian law for commercial and industrial sites. However, polygon A was comprised in the area devoted to phytoextraction in the remediation project. The sequential extraction adopted provided information on the mobility and bioavailability of metal in soil. The fractions of As non-specifically adsorbed and the specifically sorbed on mineral surfaces, were determined with $(NH_4)_2SO_4$ and KH_2PO_4, respectively.

The results also revealed a notable heterogeneity in As amounts extracted by the adopted sequential extraction. However, a very low extractable amount of As, namely potentially bioavailable, was found, which was everywhere less than 3% of the total concentration. The readily bioavailable As, extracted by $(NH_4)_2SO_4$ was rather low, with a maximum of 4.1 mg·kg^{-1} in polygon D (1064 mg·kg^{-1} of As total). However, KH_2PO_4 extracted up to 30 mg·kg^{-1} (polygon E), and its effect was generally about 30 times greater than sulfate. No linear relation was found between the total amounts and extractable arsenic.

The effectiveness of phosphate as a mobilizing agent for assisted phytoextraction in this contaminated soil was thus highlighted by the extractability tests. Phosphate is the typical mobilizing agent for As and solubilizes the As fractions specifically adsorbed on soil surfaces. Due to the high physicochemical similarity of phosphate ion and arsenate ion, phosphate moves the As adsorbed on soil constituents through a competitive exchange [33].

Although the As concentration in leachates was below the limit levels permitted by Italian law for groundwater (10 µg·L^{-1}), both in the control pots and in the phosphate-treated pots, the addition of phosphate led to a desorption of As from soil particles [34]. In fact, the As concentration values in leachates from the treated pots, even if very low, were about five fold higher than to those from the control (data non shown).

3.2. Mesocosm Experiment

3.2.1. Biomass Production

Assessing plant biomass is essential in evaluating the applicability of phytoextraction, since together with the As concentration in plants it helps define the level of pollutant removal from the soil [10]. Table 1 shows the biomass yield of each species in the various soil samples.

Table 1. Dry biomass yield (g·pot^{-1}) of *B. juncea*, *L. albus* and *H. annuus*, untreated (CT) and treated (T), grown in contaminated soils with different As concentrations.

	Plant	**Sample A**		**Sample B**		**Sample C**		**Sample D**		**Sample E**	
		Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
CT	***B. juncea***	2.49b	0.62c	2.61b	0.72c	3.42c	0.52b	2.26b	0.30b	3.52b	0.11a
	L. albus	3.20c	0.43b	0.81a	0.21a	0.71a	0.11a	1.22a	0.11a	1.82a	0.52b
	H. annuus	1.65a	0.20a	3.95c	0.31b	1.71b	0.12a	1.51a	0.11a	1.32a	0.11a
T	***B. juncea***	2.32a	0.57b	2.40a	0.41c	3.24b	0.52c	3.18b	0.46b	3.79c	0.17a
	L. albus	3.84b	0.59b	2.18a	0.34b	1.49a	0.28b	1.52a	0.14a	2.04a	0.41b
	H. annuus	2.74a	0.28a	4.59b	0.27a	1.73a	0.11a	1.44a	0.11a	2.59b	0.21a

Note: Values with different letter in the same column for control soils (CT) are statistically different at $p < 0.05$. Values with different letter in the same column for treated soils (T) are statistically different at $p < 0.05$.

Despite the differences in As concentrations among the five soil samples, the seed germination of the plants was not inhibited by the presence of the metal and the plants grew well throughout the whole experiment in all the treated and untreated soils, showing no visual signs of metal stress. Splitting the treatment over several days also helped to minimize the phytotoxic effects of As. However, the biomass production differed significantly among the plant species tested. *B. juncea* showed a high tolerance for high concentrations of As, and produced the greatest dry weight biomass even in soils with high amounts of As.

The effect of phosphate treatment on As contaminated soil was also evaluated through root and shoot dry matter production. No change in dry weight biomass of each species was detected when the phosphate was applied on the soil. However, a slight improvement in growth was observed, probably due to the fertilizing effect of phosphate and potassium added. Some studies [35,36] have demonstrated a mitigation of As phytotoxicity in the presence of phosphate nutrition. This attenuation is caused by the suppression of the high-affinity uptake phosphate co-transport system, and occurs in several arsenic tolerant species, which still maintain the ability to accumulate As [37,38].

3.2.2. Concentration of Arsenic in Plants

The amount of arsenic in plants varied according to the species. The As concentration in plants increased as a function of As concentration in the soil, especially in the aerial parts (Figure 1), where the As content ranged from 2.20 to 535 mg·kg^{-1}, with the maximum in the shoots of *H. annuus* in soil sample E. However, the plants contained a relatively low As amount and, as also reported in other studies [39,40], they accumulated the As primarily in their roots, suggesting a metal storage in the radical cells and a low mobility of the metal within the plants. For *L. albus* and *B. juncea*, the amount of As in roots was found on average up to 22 fold more than in the shoots, in the soil sample with the highest As extractable percentage (sample C), reaching up to 2600 mg·kg^{-1} of As.

In all selected species, the As concentrations in vegetal tissues increased with the application of phosphate. This highlights the potential effect of phosphate in increasing As accumulation in plants grown in As contaminated soil, due to the enhanced mobility and bioavailability of the metal in soil. Since arsenate is a phosphate analogue, in the presence of phosphorus, the arsenic adsorbed on soil surfaces is replaced [33,41], promoting a possible increase of metal uptake by plants. In fact, both ions in the radical cells of tolerant and non-tolerant plant species compete in the same transport system [5,34]. In this experiment, the phosphate effect was more pronounced in *L. albus*, particularly in samples A and B, in which the As content increased up to nine times, both in the aerial parts and in the root system. Also in *B. juncea* and *H. annuus*, after the treatments, the amount of arsenic increased in all the vegetal tissues, by about four and three times, respectively.

Regardless of the concentration present in the polygons, the plants absorbed a greater amount of As after the phosphate treatment. However, the pattern of As concentration in the aerial parts of plants compared to the total concentration in the soil was similar. In the polygons with the highest concentrations, the plants absorbed a greater amount of As, but the trend was not linear.

The transfer of inorganic ions from soil solutions to plants has been frequently interpreted as a biosorption process, and a Freundlich-like equation has been used to describe the uptake of contaminants by plants [15,42].

The Freundlich-like Equation (1) used is the same of that used for adsorption processes:

$$q = K\,C^{1/n} \tag{1}$$

However in this case, q is the contaminant concentration in plants (mg·kg^{-1}) and C is the concentration of contaminants in the soil (mg·kg^{-1}). In the Freundlich-like equation, K can be considered as the sorption capacity (a larger K indicates a larger capacity), whereas the value of $1/n$ is indicative of the strength of sorption.

Even if a Freundlich-like equation can be usefully used to study absorption of metals by plants, we have to consider that plant uptake cannot be considered a biosorption process. Biosorption is the sorption process of a contaminant by non-living biomass due to the presence of adsorbing surfaces characterized by functional groups able to interact with the contaminant. Biosorption is characterized by different processes such as adsorption on the surfaces, precipitation, ion exchange and complexation.

Plant uptake involves living plants with a physiological contaminant transport mechanism, which is dependent on the plant species. Thus, the Freundlich-like equation can be used as an operational tool for planning phytoremediation, without attributing thermodynamic significance to parameters K and $1/n$, but using them exclusively for an indication of the applicability of phytoremediation under the specific conditions of the contaminated site under examination.

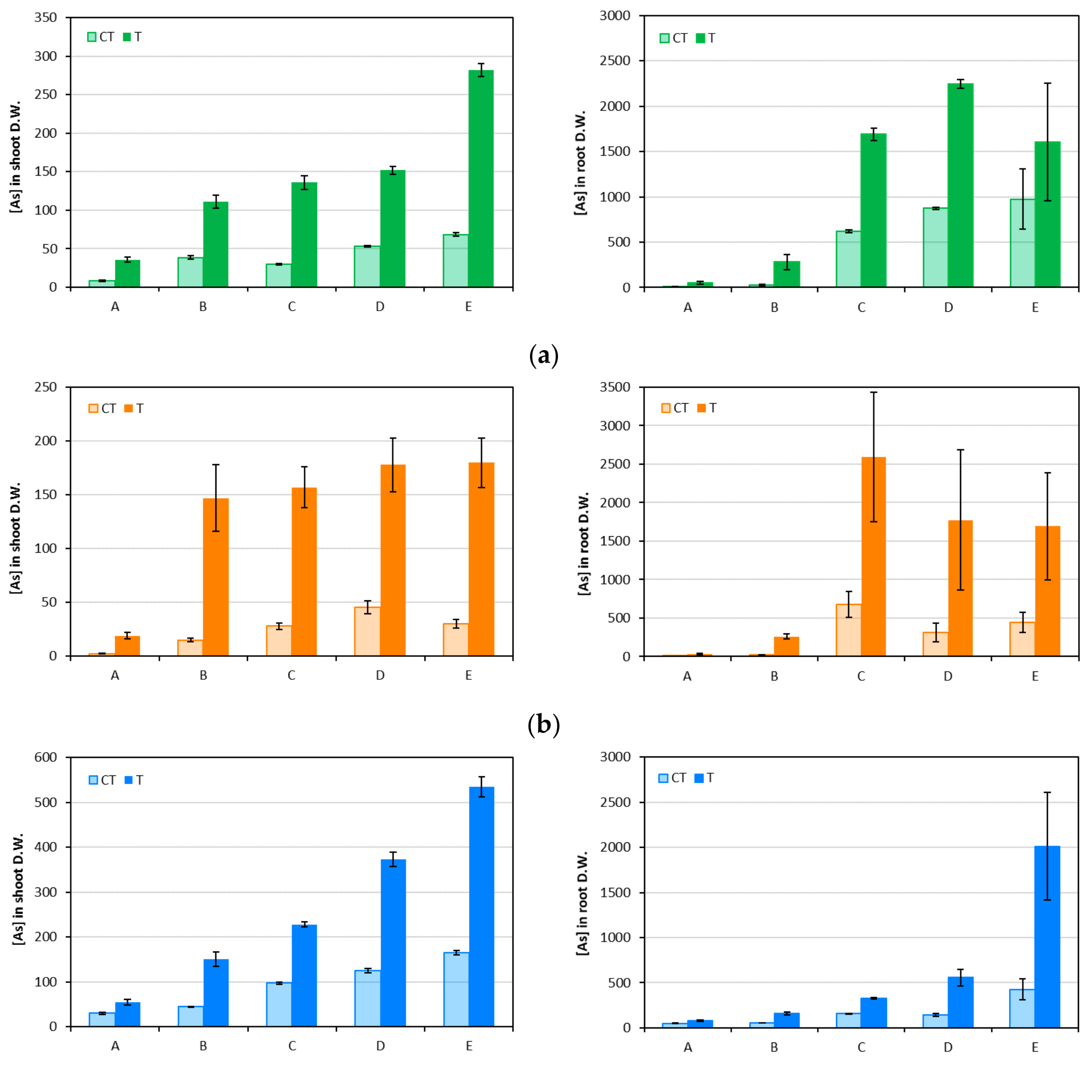
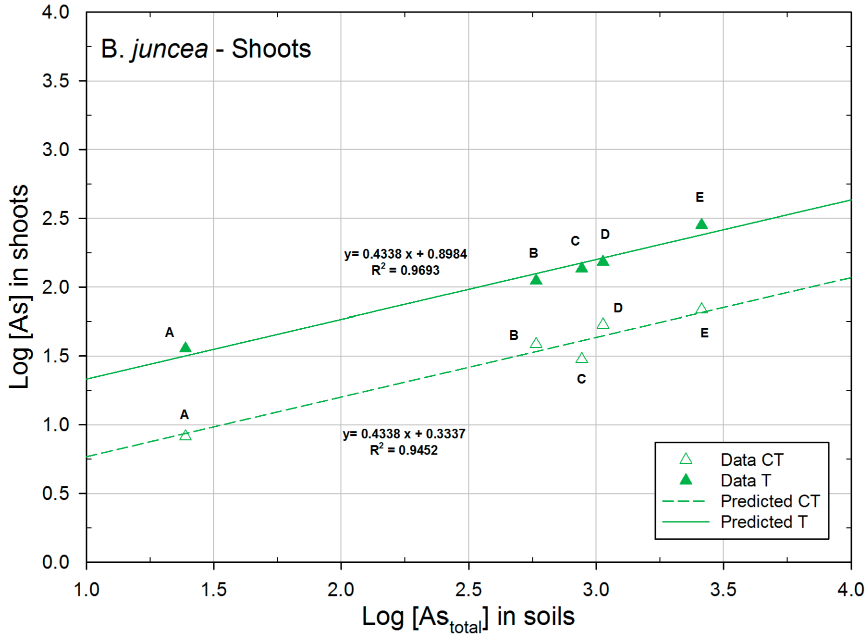


Figure 1. The effect of phosphate addition on As accumulation in shoots and roots of *B. juncea* (**a**), *L. albus* (**b**), and *H. annuus* (**c**) grown in contaminated soils with different As concentrations. Values are concentration in mg·kg^{-1} on a dry weight basis expressed as mean ± SD (n = 3).

A Freundlich-like equation can be successfully used to describe the absorption trend in relation to the concentration in the polygons (Figure 2). The Langmuir equation [43] was also tested in terms of its ability to describe the uptake from different polygons, however the results were much lower than those of the Freundlich-like model (data not shown).



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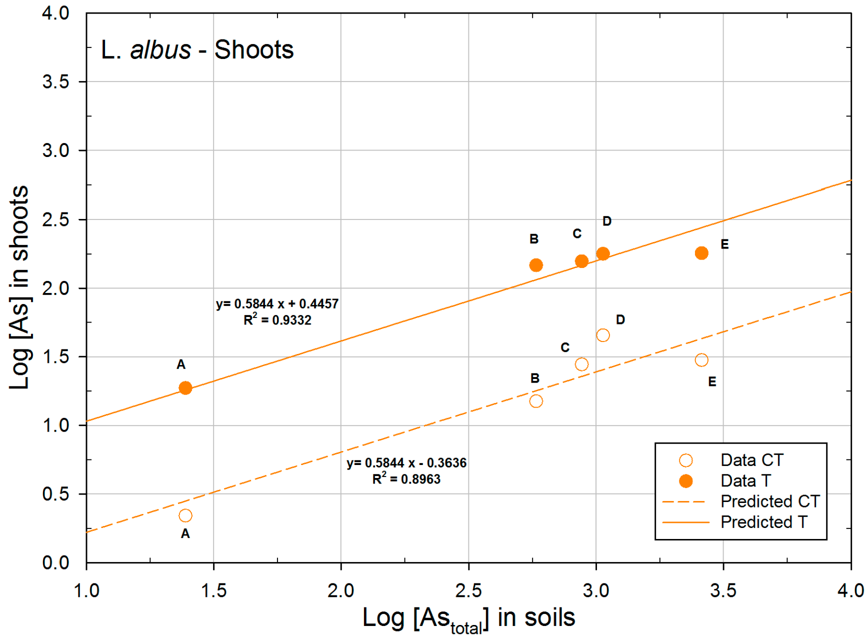

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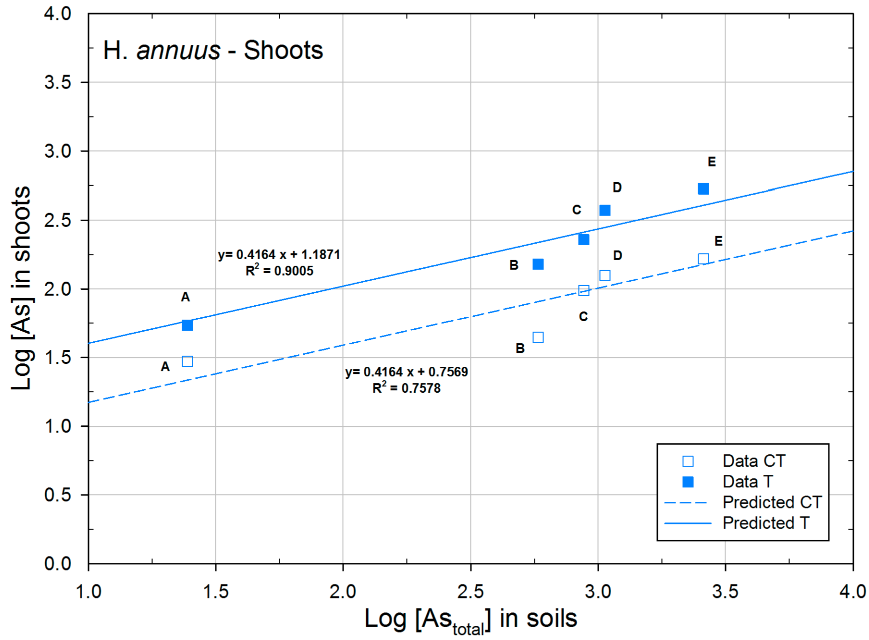
Figure 2. *Cont*.



(c)

Figure 2. Freundlich-like equation for the shoots uptake of *B. juncea* (**a**), *L. albus* (**b**), and *H. annuus* (**c**) in control (CT) and phosphate treated (T) soils.

Freundlich-like equation data are reported in Table 2.

Table 2. Relationship between shoots uptake and As concentration in soil. Parameters of the Freundlich-like model by setting $1/n$ as shared parameter.

Plant		log*K*	$1/n$	R^2
B. juncea	**CT**	0.3337a	0.4338	0.9452
	T	0.8984b	0.4338	0.9693
L. albus	**CT**	−0.3636a	0.5844	0.8963
	T	0.4457b	0.5844	0.9332
H. annuus	**CT**	0.7569a	0.4164	0.7578
	T	1.1871b	0.4164	0.9005

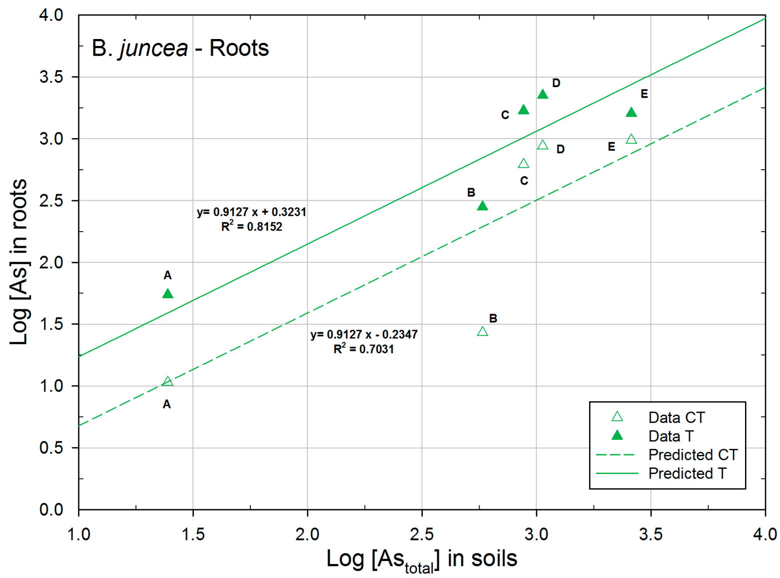
Note: Values of log*K* with different letter in the same column for each plant are statistically different at $p < 0.05$.

A Freundlich equation efficiently describes the uptake of plants in the site under study, considering the polygons with different As concentrations, with values of R^2 generally greater than 0.90. The results are in agreement with previous findings obtained, with different plant species, by Freundlich or similar models [42,44].

By operationally using the Freundlich model parameters, it can be hypothesized that the uptake capacity increased with an increasing value of K. The results show that for all the plant species the uptake always increased after phosphate treatment. Adding phosphate to the soil influenced desorption of arsenate from soil surfaces, and their release in soil solution. According to the data, the parameter $1/n$ is less than 1. This coefficient has been interpreted as an index of a plant's ability to control metal accumulation [15]. For this reason, it is reasonable to assume, for the tests with the same type of plants, the value $1/n$ as a shared parameter in the estimation process, since that parameter is closely related to the specific species under consideration, whereas it is not particularly affected by the type of treatment adopted.

Therefore considering tests conducted with the same species, with treated and untreated soils, there is a strong correlation precisely through that parameter.

When the relationship between plant concentration and total soil concentration was examined considering the root portion (Figure 3), the R^2 values of the Freundlich-like equation decreased as reported in Table 3.



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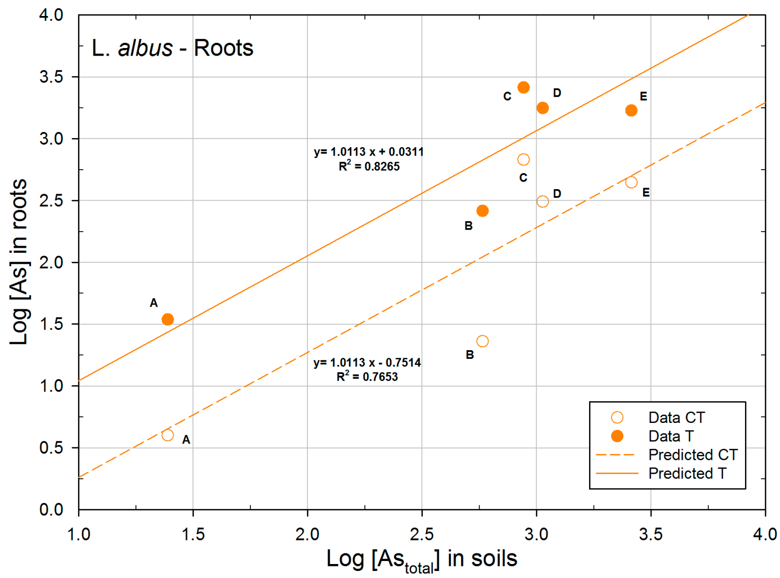
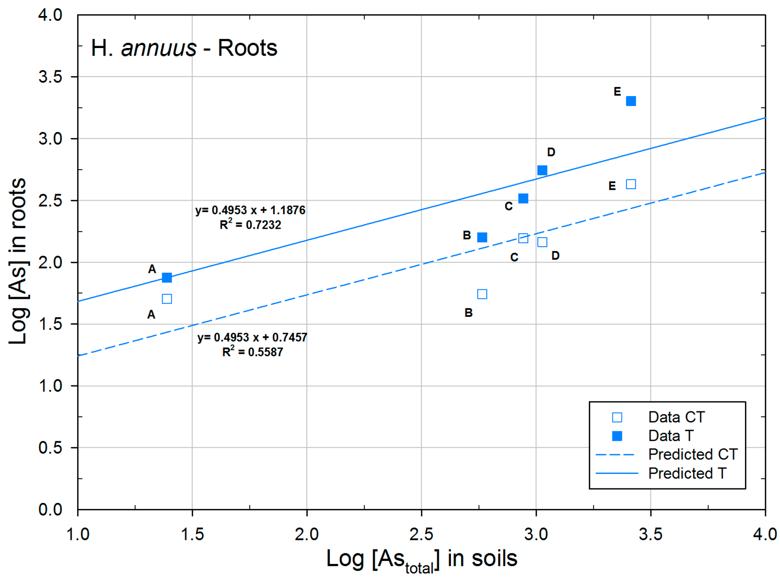


(**b**)

Figure 3. *Cont.*



(**c**)

Figure 3. Freundlich-like equation for the root uptake of *B. juncea* (**a**), *L. albus* (**b**), and *H. annuus* (**c**) in control (CT) and phosphate treated (T) soils.

Table 3. Relationship between root uptake and As concentration in soil. Parameters of the Freundlich-like model by setting $1/n$ as shared parameter.

Plant		log*K*	1/*n*	R^2
B. juncea	CT	−0.2347a	0.9127	0.7031
	T	0.3231b	0.9127	0.8152
L. albus	CT	−0.7514a	1.0113	0.7653
	T	0.0311b	1.0113	0.8265
H. annuus	CT	0.7457a	0.4953	0.5587
	T	1.1876b	0.4953	0.7232

Note: Values of log*K* with different letter in the same column for each plant are statistically different at $p < 0.05$.

Also for As concentration in the roots is confirmed the same trend that sees the value of K grow for each species downstream of phosphate treatment.

The same Freundlich-like model was also applied by correlating the amount absorbed by the plants with the potentially bioavailable metal concentration in the polygons. The results are reported in Tables 4 and 5 for shoots and roots, respectively.

Table 4. Relationship between shoot uptake and potentially available concentration of As in soil. Parameters of the Freundlich-like model by setting $1/n$ as shared parameter.

Plant		log*K*	1/*n*	R^2
B. juncea	CT	1.2418a	0.3348	0.7072
	T	1.8065b	0.3348	0.8184
L. albus	CT	0.8379a	0.4786	0.8731
	T	1.6472b	0.4786	0.7226
H. annuus	CT	1.5868a	0.3737	0.9320
	T	2.0170b	0.3737	0.8857

Note: Values of log*K* with different letter in the same column for each plant are statistically different at $p < 0.05$.

Table 5. Relationship between root uptake and potentially available concentration of As in soil. Parameters of the Freundlich-like model by setting $1/n$ as shared parameter.

Plant		log*K*	1/*n*	R^2
B. juncea	**CT**	1.5219a	0.8979	0.9566
	T	2.0796b	0.8979	0.9560
L. albus	**CT**	1.2142a	0.9705	0.9709
	T	1.9966b	0.9705	0.9635
H. annuus	**CT**	1.7300a	0.4482	0.7064
	T	2.1719b	0.4482	0.7402

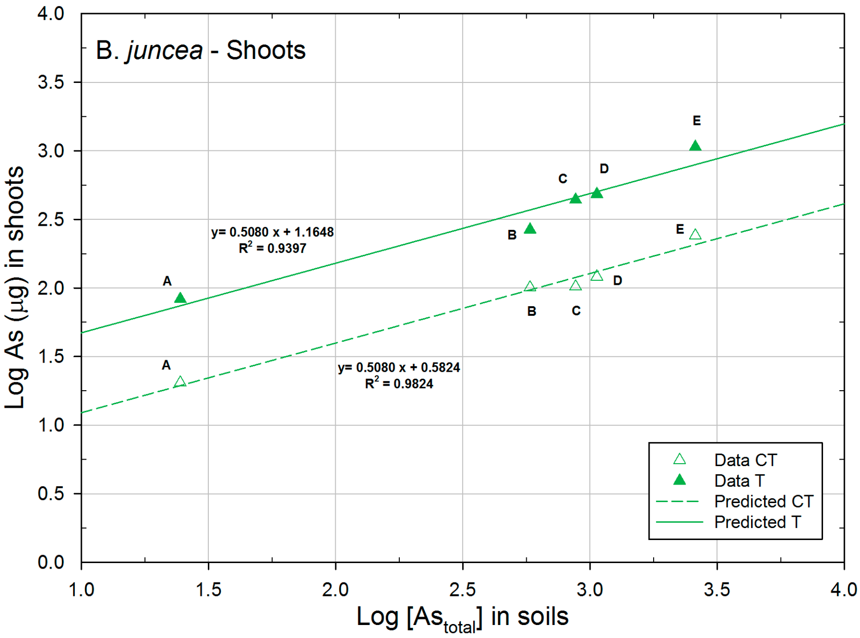
Note: Values of log*K* with different letter in the same column for each plant are statistically different at $p < 0.05$.

Also in this case, a Freundlich-like equation can be used to describe the pattern of plant uptake with respect to the potentially bioavailable concentrations in the various polygons. The K and $1/n$ coefficients changed however the trend was similar to the uptake versus total concentration, with the highest K values in the untreated and treated soils, for *H. annuus*.

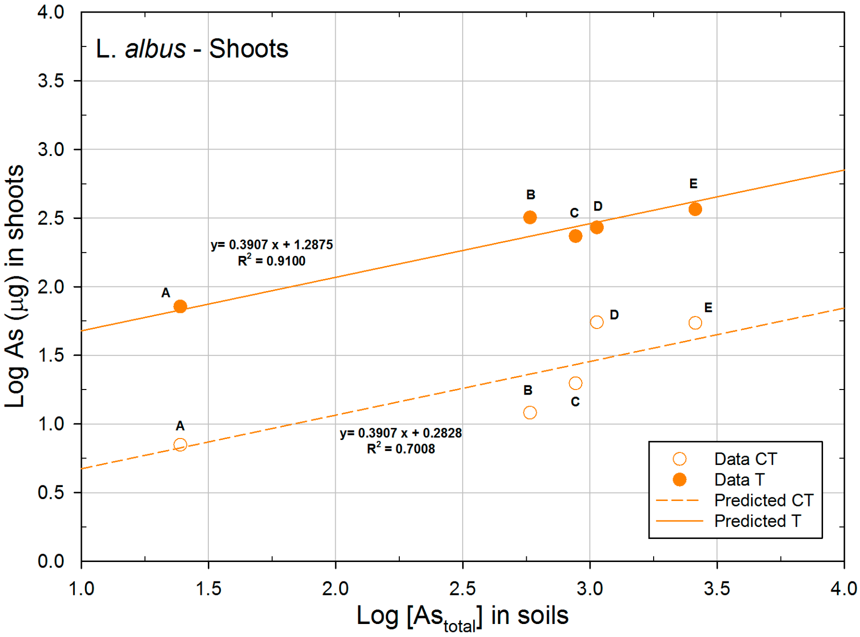
3.3. Phytoextraction Ability

In this experiment, the three coefficients, PEC, BF and TF, were also calculated to evaluate the effect of phosphate on the As bioavailability in plants growing in soil with different As concentrations. A similar trend for PEC and BF was found both in the different soils and in the different plant species. In soil samples A and B, which had lower As concentrations, the PEC and BF coefficients were found with up to 19 times higher concentrations than those in sample E. The addition of phosphate increased the PEC and BF coefficients, suggesting a higher bioavailability of As. Phosphate had the most effect in *L. albus*, where the As absorption was up to 10 times higher than in the controls. In *B. juncea* and *H. annuus*, there was an improvement after treatment. However, the effect of phosphate treatment on TF was not higher than the control. All TF values were less than 1, except in one single case. *H. annuus* was the species with highest TF mean values, ranging from 0.27 to 0.95. However no differences were found between plants grown in the various treated or non-treated soil samples. Conversely, in *B. juncea* and *L. albus* the highest TFs were found in all plants grown on A and B samples. Although the TF values were found to be low, all the species tested have a high As phytoextraction potential [27,45], and *H. annuus* was the species with the best results in terms of the soils examined.

Although the PEC and BF coefficients are useful for estimating a plant's efficiency to accumulate metals, the "total accumulation" (μg) provides a more accurate measure of the amount of metals removed from the soil by plants, and is calculated as the product of the metal concentration in vegetal tissues and the respective dry biomass yield [46]. The total accumulation pattern—related in the specific case only to the aerial part of the plants, which is the more interesting in phytoremediation evaluations—can also be correlated with the As concentration in soils by a Freundlich-like equation (Figure 4).

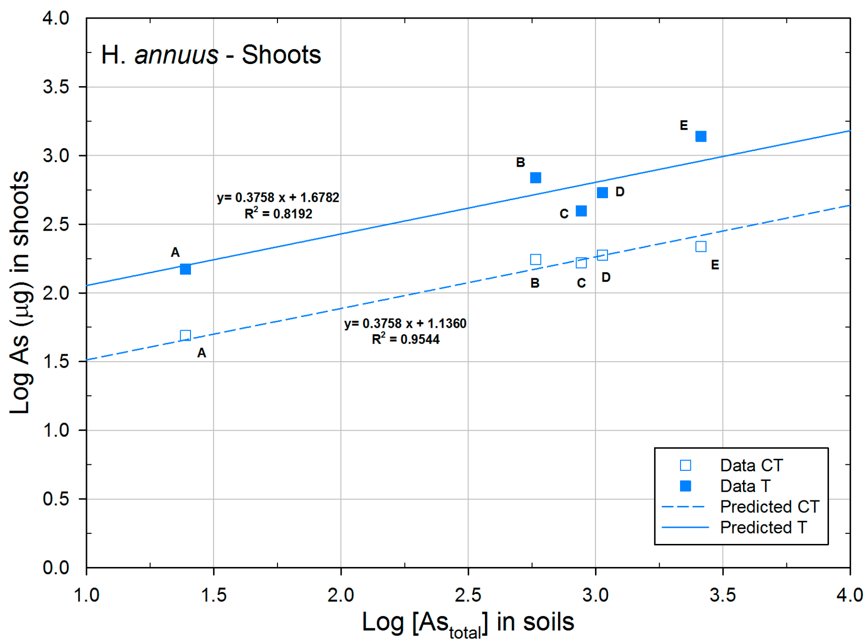


(**a**)



(**b**)

Figure 4. *Cont.*



(**c**)

Figure 4. Freundlich-like equation for the total accumulation of *B. juncea* (**a**), *L. albus* (**b**), and *H. annuus* (**c**) in control (CT) and phosphate treated (T) soils.

The results for the shoots are reported in Table 6.

Table 6. Relationship between shoot total accumulation and As concentration in soil. Parameters of the Freundlich-like model by setting $1/n$ as shared parameter.

Plant		log*K*	1/*n*	R^2
B. juncea	**CT**	0.5824a	0.5080	0.9824
	T	1.1648b	0.5080	0.9397
L. albus	**CT**	0.2828a	0.3907	0.7008
	T	1.2875b	0.3907	0.9100
H. annuus	**CT**	1.1360a	0.3758	0.9544
	T	1.6782b	0.3758	0.8192

Note: Values of log*K* with different letter in the same column for each plant are statistically different at $p < 0.05$.

The results of these mesocosm experiments also showed that phosphate might promote As total accumulation in selected species when grown in As contaminated soil. The As total accumulation of the treated plants was higher than those of the controls due to the higher As concentration in the tissues of the treated plants. The As uptake by treated plants was about 12, 4 and 3 times higher than that of the control plants, for *L. albus* and for *B. juncea* and *H. annuus*, respectively. However, *H. annuus* had the highest phytoextractive potential. The total amount of As removed from *H. annuus* treated-plants ranged from 149 to 13,800 μg, while for *L. albus,* As removal varied between 72 and 370 μg. Irrespective of phosphate treatments, the total As uptake by *L. albus* and *B. juncea* was generally higher in soil samples with the highest total As concentration, whereas no differences were revealed in *H. annuus* control plants grown in the various soil samples.

4. Conclusions

A Freundlich-like model can be used to predict the uptake of arsenic from plants in the soil of a contaminated site characterized by areas with very different contaminant concentrations. The results, based both on total and bioavailable concentrations, support the non-linearity of the uptake, and highlight the differences among plant species, thus helping in the selection of the best plant species. Many models can be used to predict the soil-to-plant transfer of elements, but often they are rather complex. A positive feature of the Freundlich-like model is that it is easy to handle, and can be used to predict the efficiency of a field-scale phytoremediation procedure. With this model, a more realistic prediction can be obtained of the potential of the technology, since the use of linear soil-to-plant transfer functions may overestimate the uptake.

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