

Article

Assessing the Influence of Marine Port Remediated Sediments on Highbush Blueberry Growth and Trace Elements Accumulation

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Abstract: The aim of this study was to investigate how biomass production and element distribution (nutrients and heavy metals) among plant organs (roots, stems, and leaves) were influenced by substrate physical and chemical properties, using acidophilic plants of *Vaccinium corymbosum* cultivars Bluecrop and Duke. A greenhouse pot experiment was conducted with highbush blueberry plants grown in an uncontaminated acidic peat-based control substrate (TS0) and two alkaline substrates enriched with remediated sediment (TS50 and TS100), characterized by high pH, Ca, and heavy metal concentrations. Both plant cultivars that were cultivated in sediment-based substrates exhibited a substantial reduction in plant growth, biomass production, and leaf chlorophyll levels. Limited translocation of microelements from belowground organs to leaves was observed across all plant samples. Cu, Fe, and Pb were predominantly accumulated in the roots of plants grown in TS-based substrates, with both cultivars acting as excluders for these metals by restricting their transport from roots to shoots. Mn and Zn were primarily retained in the stems and roots of highbush blueberry plants, with lower leaf accumulation. Notably, only Mn exhibited high translocation and bioaccumulation factor values (on average, 3.43 and 6.68, respectively), highlighting the species' strong capacity for Mn accumulation. Specifically, control plants showed significantly higher Mn concentrations than those grown in TS-enriched substrates, likely due to the acidic conditions that enhance the bioavailability of this metal and the low Ca concentration in TS0, which is known to disrupt Mn accumulation in shoots. However, this accumulation did not reach toxic levels for the plants and did not negatively impact the physiological processes of control plants, which remained particularly efficient in the Duke cv, known for its Mn resistance. This study highlights the ability of highbush blueberry plants to selectively accumulate heavy metals when grown in polluted substrates under suitable conditions, making them a valuable model for understanding metal accumulation mechanisms in the Ericaceae family.

Keywords: *Vaccinium corymbosum*; heavy metals; accumulation indices; biomass; soilless cultivation

1. Introduction

The accumulation of heavy metals in dredged sediments, originating from various human activities, has emerged as a critical environmental concern [1], significantly restrict-



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ing their potential applications in the agricultural sector. Plant tissues can accumulate both essential micronutrients vital for plant growth (such as Co, Fe, Mn, Mo, Ni, Zn, and Cu) and non-essential metals (like Pb, Cd, As, Cr, and Hg) [2,3]. However, while micronutrients are necessary in small amounts for plant functionality, non-essential metals, even in trace amounts, can detrimentally affect plant productivity and food quality. Particularly, the presence of heavy metals can alter the root structure, changing the plant accumulation capacity. In this context, many studies have revealed that a toxic element such as Cd modifies root architecture by affecting total root length, surface area, and specific root length, which in turn influences Cd absorption and translocation within the plant [4,5]. Some micronutrients, specifically Zn, can be classified as both a microelement and a risk element, depending on their availability in the soil and their concentration in plant biomass [6]. Indeed, Liu et al. [7] observed a substantial variation in cell structure, root tips, and organelles in the presence of high Zn concentrations.

It has long been recognized that the elemental composition of plant tissues is influenced by environmental conditions and by the availability of different elements in the soil [8,9]. Specifically, soil pH plays a crucial role in nutrient bioavailability, influencing plant uptake of various elements. Some elements like Al, Fe, Mn, Cu, Zn, and B are more available in acidic soil, while others like P, K, Mg, Ca, and Mo become less available as soil pH decreases. Moreover, plant uptake of metals depends on plant species, their metal specificity, growth stage, and physiological requirements. However, it is still not well known to what degree the concentrations of elements vary in different organs and how much of this variability is determined by soil chemical properties.

Certain plants exhibit specific sensitivity to heavy metal presence in soil, making them useful for bioindication purposes and phytoremediation efforts. One notable example is the wild low-growing bilberry shrub (*Vaccinium myrtillus* L.), which has proven effective in detecting environmental contamination [10–17]. Recent research has provided significant insights into the metal accumulation capabilities of different parts of *V. myrtillus*. Brašanac-Vukanovic et al. [18] demonstrated that bilberry fruits could be a valuable dietary source of essential elements for humans, especially Mn and Cu. Moreover, *V. myrtillus* has shown to be tolerant to high metal levels and has been identified as an effective accumulator of Cd, Zn, and particularly Mn, in its shoots [15,19,20].

Based on these premises, the LIFE SUBSED project (LIFE 17 ENV/IT/000347) aimed to test the cultivation of highbush blueberry (*V. corymbosum* L.), a species closely related to bilberry within the same *Vaccinium* genus, on a substrate as sediment which contains heavy metals. While bilberry grows wild in Northern Europe and in mountain areas of Southern Europe, blueberry is a widely cultivated species of economic importance due to its larger edible fruits, which are rich in anthocyanins and valuable antioxidants [21–23]. Like the wild species, blueberry prefers acidic soils ($\text{pH} \leq 5.5$), where high levels of free Mn^{2+} are available for plant absorption. However, as reported by Mora et al. [24] and Millaleo et al. [25,26], these elevated levels have a detrimental impact on plant growth and crop yields of this species. Although the plant's response mechanisms to Mn^{2+} toxicity are not well understood [27,28], a very recent study by González-Villagra et al. [29] indicates that the physiological, biochemical, and molecular characteristics of blueberry cultivars are differentially affected by Mn toxicity.

The aim of this work was to evaluate how substrate physical and chemical properties affect biomass production, elemental concentration in plant organs, and bioaccumulation factors of two highbush blueberry cultivars grown in heavy metal-rich sediments (TS). Specifically, the study determined the contents of thirteen elements categorized into major (P, Ca, Mg, K, and Na) and trace elements (Cu, Ni, Fe, and Mn, considered as microelements,

and Cd, Cr, Pb, and Zn, considered as risk elements) in the roots, stems, and leaves of blueberries, as well as in the investigated substrates.

2. Materials and Methods

2.1. Plant and Soil Material

Certified two-year-old blueberry plantlets cvs Duke and Bluecrop were planted in 35 L plastic pots and conventionally grown under greenhouse conditions. The cultivation trial was performed at the Az. Agricola Franceschini (Central Italy, 43°51'32.1" N 10°41'11.3" E) during Spring 2020 and Autumn 2021. The control substrate (TS0) was composed of a blend of 60% peat Brill® 1 and 40% volcanic pumice (*v/v*) sourced from the Brill Substrate (Georgsdorf, Germany) and Europomice S.r.l (Milano, Italy), respectively. The same type of substrate blend enriched with a ratio of 50% of a remediated sediment (TS50) and the remediated sediment alone (TS100) were used as the polluted substrates. The remediated sediment (TS) originated from a marine sediment dredged from Leghorn Port (Central Italy, 43°33'25" N, 10°17'39" E), which had undergone phytoremediation and had been subjected to three months of landfarming according to Macci et al. [30]. Marine sediment is a waste material that is challenging and costly to manage. In particular, the dredged sediment from Leghorn Port was chosen for its proximity to a key area of the flower and ornamental plant industry, which requires large quantities of substrates for soilless plant production. It exhibits a high bulk density, an alkaline pH, and a silty-clay texture [30]. Pots were arranged in three blocks, each consisting of 3 pots per substrate × cultivar, for a total of 54 pots (3 substrates × 2 cultivars, replicated in 3 blocks, each consisting of 3 plants).

Drip irrigation was used to control the water supply, and all plants received 480 cc of water per day from the same reservoir using six drip emitters per pot at a flow rate of 80 cc min⁻¹, and irrigation time of 1 min per day. The pH level of the irrigation water was maintained between 5.5 and 6.0, while electrical conductivity ranged from 0.42 to 0.57 dS m⁻¹. The plants were fertigated once per week with FINISHER water-soluble fertilizer, NPK 15:7:30 + 3MgO + TE (UralChem, Moscow, Russia) in the range of 200 g per plant in the periods of May–June and September–April and 400 g per plant from July to August.

2.2. Physical and Chemical Substrate Characterization

The substrate samples were collected one day before planting and at the end of the experiment in November 2021. The samples were first analyzed for physical and chemical parameters (bulk density, pH, electrical conductivity, total N, total organic C, NH₄⁺, and NO₃⁻), using approx. 500 g of air-dried, milled, sieved, and homogenized substrate samples, according to Tozzi et al. [31]. Thereafter, the content of macronutrients (Ca, K, Mg, Na, and P) and heavy metals (Cr, Cu, Fe, Mn, Ni, Pb, Zn, and Cd) were determined by ICP (5900 ICP-OES; Agilent, Santa Clara, CA, USA) after acid digestion in a microwave (Ethos 1; Milestone S.r.l, Bergamo, Italy) with hydrogen peroxide–nitric acid H₂O₂/HNO₃ 1:3 *v/v*. Total P was determined by using a colorimetric method [32] after acid extraction.

2.3. Plant Growth and Ecophysiological Parameters

Blueberry vegetative growth (plant height, trunk diameter, and number of new sprouts) was monitored (during the cultivation at 5–6-month intervals) in November 2020, May 2021, and November 2021, and for each assessment, the cumulative growth and number of developed shoots were considered.

Destructive plant analyses were performed at the end of the experiment in November 2021. Fresh biomass was air-dried at 75 °C until it was completely desiccated, and the dry matters of plant roots (RDM), stems (SDM), and leaves (LDM) were measured. Subse-

quently, plant samples were ground using a stainless-steel mill for further analysis. Prior to air drying, the total leaf area per plant was measured with an electronic leaf area meter (WinDIAS Image Analysis System, Delta-T Devices, Cambridge, UK).

SPAD measurements were taken 416 days after transplanting (DAT) (on 28 June 2021), within a 2 h window across solar noon (between 11:00 and 13:00) using a handheld dual-wavelength chlorophyll meter, Minolta SPAD-502 (Minolta Camera Co., Ltd., Osaka, Japan). For each cultivar x substrate combination, five fully expanded leaves from the upper part of the plant were selected. SPAD readings were taken on each side of the midrib of leaves, which have been cleaned of any surface dust. Care was taken to ensure that the SPAD meter sensor completely covered the leaf lamina without interference from veins or midribs. The instrument stored and automatically averaged the readings to generate a single reading per plot.

Colorimetric analyses were performed on leaf samples collected immediately after the SPAD readings were taken (416 DAT) using the Chromameter, Minolta CR 200 colorimeter (XRITE, SP64, Grand Rapids, MI, USA) on three different blade points. The chroma index was calculated using the coordinates a^* and b^* ($\text{Chroma} = [(a^{*2} + b^{*2})^{(1/2)}]$), where a^* represents redness and b^* represents yellowness.

2.4. Analysis of Macro Elements and Metal Concentration in the Plant Samples

At the end of the growing cycle, samples from the roots, stems, and leaves were collected to determine the heavy metal content in the plants. Throughout the experimental period, the plants were meticulously cared for, with other stressors eliminated and consistent temperature, humidity, and lighting conditions maintained for all variants and replicates.

Nutrients and heavy metals were analyzed following official UNI EN ISO analytical protocols. For the determination of nutrient and heavy metal concentrations, 500 mg of each pulverized dried material was mineralized by microwave-assisted acid digestion (Ethos 1, Milestone) using 25 mL of $\text{H}_2\text{O}_2:\text{HNO}_3$ 1:3 (v/v) and analyzed by ICP (iCAP 7000 Plus ICP-OES; Thermo Fisher Scientific, Waltham, MA, USA). Blanks were prepared for each analytical batch to account for potential metal contamination of reagents and reactors [33–35].

The accumulations of nutrient elements and trace elements (ANEs and ATEs) in the studied plants were analyzed following the methodology described in [36]. To determine the total amount of elements, the measured values were converted into equivalents. The sum of the elements, expressed in $\text{mmol}_c \text{ kg}^{-1}$, was calculated using the following equation:

$$Y = \sum_{i=1}^i \frac{Z}{z}$$

where Z represents the element content in mg kg^{-1} , and z corresponds to the atomic mass divided by the ion valence. Expanding this equation for specific elements:

$$Y = \frac{Ca}{40.1} + \frac{K}{39.1} + \frac{Mg}{24.3} + \frac{Na}{23.0} + \frac{P}{31.0} + \frac{Cr}{52.0} + \frac{Cu}{63.5} + \frac{Fe}{55.8} + \frac{Mn}{54.9} + \frac{Ni}{58.7} + \frac{Pb}{207.2} + \frac{Zn}{65.4}$$

After computing the total accumulation, the percentage contribution of each element (X) was determined using the following:

$$X_z = \frac{\left(\frac{Z}{z}\right)}{Y} \times 100$$

2.5. Data Analysis and Accumulation Indices

Substrate metal contamination and the plant's capacity to accumulate and translocate metals from the roots to the harvestable parts were assessed by calculating some specific indices (Table 1) based on the metal content. In more detail, the transfer coefficient (TC), translocation factor (TF), bioaccumulation factor (BF), enrichment factor (EF), removal efficiency (RE), and metal uptake (MU) were determined in both substrates (TS0, TS50, and TS100) and plant organs (root, stem, and leaf) collected in November 2021.

Table 1. Accumulation indices used to assess the highbush blueberry capacity to uptake metal ions from the substrate and to accumulate and translocate them from the roots to the aboveground plant parts.

Accumulation Indices	Abbreviation	Formula	Meaning	References
Transfer coefficient	TC	Heavy metals in roots/heavy metals in soil (mg/kg^{-1} DW)	A TC greater than 1 denotes metal accumulation, a TC around 1 means no influence by the metal, and a TC less than 1 indicates minimal metal uptake by the plant	[37]
Translocation factor	TF	Heavy metals in the upper aerial portion of the plant (leaves + stems)/heavy metals in the roots (mg/kg^{-1} DW)	Values greater than 1 indicate effective translocation of metals from the roots to the aboveground parts of the plant	[38]
Bioaccumulation factor	BF	Heavy metals in aboveground plant tissues/heavy metals in soil (mg/kg^{-1} DW)	Values are usually less than 1 for metal exclusion species, while greater than 1 for metal accumulator species	[39]
Enrichment factor	EF	Heavy metal concentration in the contaminated soil-plant/heavy metal in the uncontaminated soil-plant (mg/kg^{-1} DW)	EF values greater than 1 indicate higher metal availability and distribution in the contaminated soil, thus increasing metal accumulation in plants grown on such soil relative to their reference values.	[40]
Removal efficiency	RE	$100 \times (\text{initial heavy metal concentration in soil} - \text{final heavy metal concentration in soil}) / \text{initial concentration of heavy metal in soil}$ (%)	High percentage values indicate hyperaccumulation characteristics of the species	[41]
Metal uptake	MU	Metal concentration in a shoot or root \times dry weight of the shoot or root (mg kg^{-1} dw)	High values, when paired with high biomass, suggest potential for metal bioextraction, while low values indicate exclusion by restricting metal transport	[42]

TC indicates the plant's ability to accumulate metals relative to the soil's metal concentration. TF shows the ability of the plant to move the metal from the roots to the stems and leaves. BF serves as an indicator of a plant's capacity to accumulate heavy metals in aboveground plant tissues. EF assesses the level of soil pollution and metal accumulation in plants growing on contaminated soil compared to those on control soil. RE denotes the plants' capacity to extract a specific quantity of metal ions from the soil over a designated time span. Finally, MU provides insight into the plant's capacity to accumulate heavy metals in proportion to its dry biomass.

2.6. Statistical Analysis

Once normal distribution was confirmed, experimental data concerning plant growth, ecophysiological parameters, and nutrient and metal content were analyzed by ANOVA.

Mean comparisons were conducted using Tukey's test, with statistical significance set at 99% ($p < 0.01$), using SPSS v27 software (SPSS Inc., Chicago, IL, USA), using three replicates per treatment.

3. Results

3.1. Substrate Physical and Chemical Properties

The physicochemical properties of the three tested substrates were determined before blueberry cultivation (on 8 May 2020; t0) and after harvesting (on 15 November 2021; tf). The mixtures' characteristics are reported in Table 2, while the interactions between substrates and the cultivars (after harvesting) are described in Table S1. Both sediment-based substrate samples (TS50 and TS100) had basic pH and higher mineral levels compared to peat, while organic matter content and total nitrogen were much lower than the control sample.

Table 2. Physicochemical properties, macronutrients, and heavy metal concentrations of substrate samples before planting (t0) and at the end of the experiment (tf).

Parameters	Unit	Substrate Composition						
		TS0	TS50	TS100	TS0	TS50	TS100	
		t0			tf			
Physicochemical characteristics	Bulk density	g cm ⁻³	0.35 c	0.61 b	1.03 a	0.40 c	0.78 b	1.03 a
	pH		4.59 c	6.72 b	7.81 a	3.72 c	5.39 b	6.56 a
	EC	dS m ⁻¹	0.15 c	0.23 b	0.27 a	0.76 a	0.31 c	0.41 b
	NO ₃ ⁻	mg kg ⁻¹	42.88 a	28.49 b	45.14 a	79.58 a	51.64 c	61.05 b
	NH ₄ ⁺		11.40 a	6.73 b	0.61 c	19.26 a	1.34 b	1.24 b
	TN	%	0.63 a	0.14 b	0.08 c	0.65 a	0.12 b	0.10 b
	TOC		7.96 a	3.52 b	0.71 c	15.31 a	3.02 b	1.29 b
Macronutrients	P		302.45 b	388.85 a	382.18 a	600.40 a	558.70 b	527.58 b
	Ca		4819.98 c	25,728.67 a	24,040.00 b	6261.70 c	10,046.44 b	11,613.37 a
	Mg	mg kg ⁻¹	1990.66 b	5143.08 b	5758.00 a	2204.27 b	3380.83 a	3326.89 a
	K		5690.61 a	3036.42 b	1949.00 c	7329.58 a	3691.17 b	3411.13 b
	Na		1318.39 b	1873.15 a	1349.00 b	2416.03 a	912.95 b	717.51 c
Heavy metals	Cr		5.24 c	51.00 b	58.50 a	2.50 c	47.55 b	54.31 a
	Fe		5928.98 c	15,066.99 b	17,408.00 a	4995.22 b	12,979.44 a	12,724.48 a
	Mn		341.59 b	387.88 a	396.63 a	56.75 c	118.63 b	209.66 a
	Ni	mg kg ⁻¹	6.51 c	37.62 b	44.00 a	3.20 b	30.99 a	36.89 a
	Pb		23.00 c	43.07 b	46.00 a	17.76 b	33.92 a	34.82 a
	Cu		73.37 c	102.13 b	127.32 a	45.00 a	40.82 b	12.10 c
	Zn		80.50 c	178.00 b	185.00 a	49.40 b	144.98 a	149.94 a

(EC) Electrical conductivity. Total nitrogen (TN). Total organic carbon (TOC). Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different at $p < 0.01$. The values relating to the substrate samples at the final time (tf) are the means of the Bluecrop and Duke cultivars.

A significant variation in bulk density was observed with the addition of sediments in the substrates. Considering the t0, TS0 (control) revealed the lowest bulk density (0.35 g cm⁻³), whilst TS100 revealed the highest one (1.03 g cm⁻³), which exceeded the limit set by the Italian L.D. 75/2010 [43]. However, TS100 bulk density was the only parameter that did not comply with L.D. 75/2010 [43]. A similar trend (TS100 > TS50 > TS0) was observed for both pH and EC, with TS0 showing the minimum values (4.59 and 0.15 dS m⁻¹, respectively) and TS100 the maximum ones (7.81 and 0.27 dS m⁻¹, respectively). On the contrary, TS0 values for NH₄⁺, TN, and TOC (11.40 mg kg⁻¹, 0.63% and 7.96%, respectively) were significantly higher than both TS50 (6.73 mg kg⁻¹, 0.14% and 3.52%, respectively), and TS100 (0.61 mg kg⁻¹, 0.08% and 0.71%, respectively).

The macro element concentrations varied significantly among the tested substrate blends. Prior to planting, sediment-enriched substrates exhibited higher levels of P, Ca, Mg, and Na, while K levels decreased as the TS content increased. Following 1.5 years of blueberry growth in these same substrate blends, Ca, Mg, and Na levels increased in the control substrate (TS0) while decreasing in TS50 and TS100 blends. Meanwhile, P and K levels were found to increase across all substrate blends.

Significant differences were observed between the metal concentration from TS-based substrates and the control soil both in May 2020 (t0) and in November 2021 (tf). Initial analyses showed that there was a clear difference between the concentration of heavy metals such as Cu, Fe, Zn, Pb, Cr, Mn, and Ni in the investigated substrates. These levels showed an increasing trend with the rise in the proportion of sediment, except for Mn, which exhibited similar levels in both TS50 and TS100 samples. The Ni concentration surged nearly 6–7 times in the TS50 and TS100, while Cr exhibited an even greater increase of approximately 10–11 times. However, concentrations of Cu, Pb, and Ni remained below the alert threshold established by Italian regulation for contaminated sites [44] in both TS50 and TS100 despite a higher concentration of zinc, which exceeded the limits of the intervention value (Table 2). Aluminum and cadmium were undetectable in both TS-containing substrates and the control.

After harvesting, in November 2021, Cu, Fe, Mn, and Pb levels substantially decreased in all substrates. The concentrations of Cr, Zn, and Ni revealed a substantial reduction in the control (~50%) while showing a less significant decrement in TS-enriched substrates.

Some differences among the cultivars also emerged, as shown in Table S1, with cv Bluecrop showing lower values of both macro and trace elements in the control substrate. In more detail, a substantial decrease in K content (~40%) was observed in TS0 in Bluecrop when compared with Duke. A similar trend was noted in Mn and Pb concentrations in the control substrate, with cv Bluecrop showing a reduction of 59% and 40%, respectively.

3.2. Plant Development and Growth

The blueberry plants grown on the control substrate had a consistently greater development than the blueberry from the TS-based substrates (Table 3). Specifically, the diameter measured at the base of the collar after 1.5 years of cultivation in the control plants was found to be 26.33 mm, compared to 20.0 and 18.8 mm measured on plants grown on substrates containing 50 and 100% TS, respectively. The height of the plant cultivated in the peat substrate (122.83 cm) was almost 40% higher than that of the blueberries from TS50 (75.83 cm) and TS100 (74.33 cm), which were also being more vigorous. Similarly, the number of new sprouting shoots of plants grown on the control was 28.00, while shoots of the blueberry from the TS substrates were between 18.50 and 21.50. The Bluecrop cv was more affected by the negative substrate effect than the Duke cv for all considered parameters (height, trunk diameter, and new sprouts), showing a reduction ranging between 23 and 28%.

Measurements performed on blueberry leaf area index (LAI) revealed that LAI decreased with increasing TS percentage (Table 4). The dry matter content of plant roots and aboveground biomass was lower in TS-based substrates and further diminished as the proportion of sediment in the substrate increased. Among the cultivars, Duke exhibited higher root and aboveground dry matter compared to Bluecrop, regardless of whether they were grown in pure peat or substrates containing TS.

Table 3. Effect of substrate composition and cultivar growth parameters of potted highbush blueberry.

Factor	Height (cm)	Trunk Diameter (mm)	New Sprouts (n)
Substrate (Su)			
TS0	122.83 a	26.33 a	28.00 a
TS50	75.83 b	20.00 b	21.50 b
TS100	74.33 b	18.80 b	18.50 b
Cultivar (Cv)			
Bluecrop	77.11 b	18.56 b	18.94 b
Duke	100.22 a	24.89 a	26.39 a
Su × Cv			
TS0 × Bluecrop	115.80 b	25.00 a	23.56 b
TS0 × Duke	129.70 a	27.67 a	32.44 a
TS50 × Bluecrop	66.00 e	16.000 c	17.50 c
TS50 × Duke	91.33 c	25.30 a	25.50 b
TS100 × Bluecrop	60.30 f	14.67 c	15.78 c
TS100 × Duke	82.67 d	21.70 b	21.22 bc

Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different at $p < 0.01$.

Table 4. Effect of substrate composition and cultivar on plant biomass dry matter and leaf area of potted highbush blueberry.

Factor	Root DM (g)	Aboveground DM (g)	Total DM (g)	Leaf Area (cm ²)
Substrate (Su)				
TS0	403.15 a	450.60 a	853.75 a	19.1 a
TS50	149.54 b	164.96 b	314.50 b	16.7 b
TS100	122.78 c	136.03 c	258.81 c	15.2 c
Cultivar (Cv)				
Bluecrop	211.99 b	225.08 b	437.07 b	16.8 b
Duke	238.32 a	275.98 a	514.30 a	17.1 a
Su × Cv				
TS0 × Bluecrop	387.06 a	414.06 b	801.12 b	19.0 a
TS0 × Duke	419.23 a	487.14 a	906.37 a	19.2 a
TS50 × Bluecrop	135.44 c	138.74 d	274.18 d	16.5 c
TS50 × Duke	163.64 b	191.18 c	354.82 c	16.9 b
TS100 × Bluecrop	113.47 d	122.43 e	235.90 e	15.1 d
TS100 × Duke	132.08 c	149.62 d	281.70 d	15.2 d

Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different at $p < 0.01$.

3.3. SPAD Readings and Leaf Color

The experimental data collected for leaf chlorophyll and leaf color are reported in Table 5. The presence of the treated sediment in the substrate (TS50 and TS100) significantly reduced SPAD chlorophyll (43.99 and 35.96, respectively) compared with plants grown on the standard peat substrate (55.53). Moreover, the color of the blueberry leaves planted in the TS100 (25.84) was intensified to light green with a more pronounced yellow component than that of the leaves of the control sample (20.99). Conversely, no significant variations were observed between TS50 and TS0. Some differences were detected between the cultivars as well, with Bluecrop showing a lower SPAD value on average (39.19) than Duke one (51.10).

Table 5. Effect of substrate composition and cultivar on SPAD readings and leaf color of potted highbush blueberry.

Factor	SPAD	Chroma Index
Substrate (Su)		
TS0	55.53 a	20.99 b
TS50	43.99 b	22.46 b
TS100	35.96 c	25.84 a
Cultivar (Cv)		
Bluecrop	39.19 b	23.95 ns
Duke	51.10 a	22.25 ns
Su × Cv		
TS0 × Bluecrop	38.93 bc	20.95 ns
TS0 × Duke	72.14 a	21.04 ns
TS50 × Bluecrop	43.31 b	23.67 ns
TS50 × Duke	44.49 b	21.26 ns
TS100 × Bluecrop	35.34 c	27.24 ns
TS100 × Duke	36.66 c	24.45 ns

Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different at $p < 0.01$. ns = non-significant.

3.4. Plant Elemental Composition and Translocation Pattern

The bioaccumulation of the studied elements in root, stem, and leaves of Bluecrop and Duke cvs was evaluated after 18 months of soilless cultivation. The obtained element concentration in the different parts of the plant is shown in Tables S2 and S3.

Sediments influenced blueberry's macronutrient content in different plant organs (Table S2). In this context, the increase in sediment concentration (TS50 and TS100) led to an increment of Ca and Na in all the plant parts. Conversely, a decrease in K was observed in sediment-rich substrates in comparison with the control in stem and root.

The mean values of heavy metal concentration in *V. corymbosum* were found in descending order: Mn > Fe > Zn > Cu > Ni > Cr and Pb. Overall, there was a clear increase in the concentration of the studied metals in plant organs (i.e., leaf, stem, and root) collected from TS50 and TS100, except for Mn, which had the highest content in plants from TS0. Additionally, Bluecrop accumulated higher levels of each metal compared to Duke (Table S3).

The concentrations of Fe, Cu, Cr, and Pb were higher in the roots compared to other plant parts, with the highest root contents observed in cv Bluecrop collected from TS50 for Fe and Cu (613.0 and 7.7 mg kg⁻¹, respectively) and from TS100 for Cr and Pb (2.24 and 1.5 mg kg⁻¹, respectively). On the other hand, Zn and Mn were highest in blueberry stems, and specifically, Mn levels (758 and 690 mg kg⁻¹ in Bluecrop and Duke, respectively) were notable in control plants, while no discernible variations were observed among substrates for Zn levels which were higher in Bluecrop (29.50–31.00 mg kg⁻¹) than in Duke (20–23 mg kg⁻¹). Finally, Ni content was accumulated in greater amounts in the leaves of all samples.

The accumulation of the nutrient and trace elements (ANEs and ATEs) in the *V. corymbosum* plant tissue was calculated separately for the macro elements and heavy metals. The average level of accumulated macronutrients did not differ substantially among the plant tissues of blueberries obtained from the control or from TS-based substrates (Table 6).

However, the percentage composition of the elements varied significantly. In more detail, an increase in Ca and Na proportion was observed in sediment-rich substrates, whilst P and K contents were higher in the control. No substantial divergences were noted in Mg levels. A nearly two-fold ATE value in control plants (18.66 mmol_c kg⁻¹) was primarily due to Mn (Table 7). Indeed, the percentages of Fe, Zn and Cu (38.53, 10.02 and 1.25%, respectively) in plants from TS100 were consistently higher than those in blueberries collected from the control (8.75, 3.42, and 0.54%, respectively).

Table 6. Accumulation of nutrient elements (ANEs) in highbush blueberry shoots.

Substrate	Ca (%)	K (%)	Mg (%)	Na (%)	P (%)	ANE mmol _c kg ⁻¹
TS0	8.95	22.64	8.49	4.66	55.26	1371.83
TS50	14.60	21.60	8.04	5.46	50.30	1564.94
TS100	17.32	18.16	8.77	10.65	45.11	1712.83

Table 7. Accumulation of trace elements (ATEs) in highbush blueberry shoots.

Substrate	Cr (%)	Cu (%)	Fe (%)	Mn (%)	Ni (%)	Pb (%)	Zn (%)	ATE mmol _c kg ⁻¹
TS0	0.03	0.54	8.75	87.02	0.24	0.00	3.42	18.66
TS50	0.07	0.86	21.07	71.13	0.51	0.01	6.34	10.32
TS100	0.12	1.45	38.53	48.95	0.88	0.04	10.02	6.91

The mean values of the accumulation indices (TC, TF, BF, EF, RE, and MU) for highbush blueberries are reported in Table 8. Depending on the metal type and plant organ, different values of transfer and translocation factors were obtained. The TC values were generally below 1 except for the bioaccumulation of Mn in roots from TS0, which was higher than 1 compared to the TS-enriched substrates (9–63 fold higher).

Table 8. Accumulation indices in highbush blueberry.

Substrate	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Transfer coefficient (TC)							
TS0	0.05	0.09	0.02	9.47	0.15	0.03	0.34
TS50	0.01	0.15	0.03	1.21	0.03	0.02	0.13
TS100	0.03	0.37	0.02	0.15	0.02	0.03	0.09
Average	0.03	0.20	0.03	3.61	0.06	0.03	0.19
Translocation factor (TF)							
TS0	2.25	1.63	0.75	1.66	5.72	0.24	2.45
TS50	0.56	0.95	0.30	2.81	3.51	0.41	2.32
TS100	0.30	1.44	0.52	5.81	5.11	0.53	3.21
Average	1.04	1.34	0.52	3.43	4.78	0.39	2.66
Bioaccumulation factor (BF)							
TS0	0.11	0.14	0.02	15.74	0.84	0.01	0.84
TS50	0.01	0.14	0.01	3.40	0.10	0.01	0.29
TS100	0.01	0.53	0.01	0.89	0.10	0.02	0.30
Average	0.04	0.27	0.01	6.68	0.35	0.01	0.48
Enrichment factor (EF)							
TS0	1	1	1	1	1	1	1
TS50	1.43	0.88	1.33	0.45	1.16	2.77	1.03
TS100	1.65	0.98	1.63	0.21	1.34	5.09	1.09
Average	1.54	0.93	1.48	0.33	1.25	3.93	1.06
Removal efficiency (RE)							
TS0	52.24	38.67	15.75	83.39	50.84	22.80	38.63
TS50	6.77	60.03	13.86	69.42	17.62	21.23	18.55
TS100	7.16	90.50	26.90	47.14	16.15	24.30	18.95
Average	22.06	63.06	18.84	66.65	28.21	22.78	63.06
Metal uptake (MU) roots							
TS0	0.05	1.61	48.98	216.75	0.19	0.18	6.83
TS50	0.10	0.90	60.34	21.46	0.13	0.11	2.74
TS100	0.18	0.55	35.42	3.93	0.09	0.13	1.72
Average	0.11	1.02	48.25	80.71	0.14	0.14	3.77
Metal uptake (MU) shoots							
TS0	0.12	2.93	41.18	402.39	1.21	0.05	18.68
TS50	0.06	0.94	20.08	66.60	0.52	0.05	7.01
TS100	0.06	0.87	20.27	25.29	0.49	0.08	6.12
Average	0.08	1.58	27.18	164.76	0.74	0.06	10.60

A translocation factor (TF) above 1 was observed for Cu, Mn, Ni, and Zn across all substrates. The exception was Cr, where $TF > 1$ was only found in the control substrate, while for Fe and Pb, the TF was consistently below 1. Regarding Mn, TS-enriched substrates revealed a greater TF than TS0 (+2–3.5 fold). In addition, blueberry plants resulted in high Mn accumulators, as described by the BF parameter, which was >1 in all substrates, except for TS100 (0.89). In this context, plants grown in TS0 showed a substantial increase (5–15 fold) in BF compared to what was observed in TS-enriched media.

The highest EF values for Cr, Cu, Fe, Ni, Pb, and Zn were detected for highbush blueberries for TS100, and the highest EF for Mn for TS50. In particular, TS100 had a high EF value for Pb, being 2 and 5 times greater than TS50 and TS0, respectively.

Large variations in the RE of Cr, Mn, and Ni were observed among the different substrates. However, the reduction in the amount of these elements did not depend on the level of substrate pollution. Indeed, plants grown in TS0 displayed higher RE values than plants obtained on TS-enriched substrates, with values up to 52.2%, 60.0%, and 43.1% for Cr, Mn, and Ni, respectively. The decrease in Cu, Fe, Pb, and Zn showed values that were more consistent across the various substrates, ranging between 60.0 and 83.5%, 13.9 and 26.9%, 21.2 and 24.3%, 62.2, and 69.1%, respectively.

In addition, a significantly higher uptake (MU) of Mn and Zn was found in the roots of highbush blueberries grown in TS0 (control) compared to sediment-rich substrates. Conversely, TS50 and TS100 revealed a greater MU in roots for Cr (+50–72%) than the control. The shoot Mn, Ni, and Zn amounts were significantly higher compared to the values of the roots. With the sole exception of Pb, the highest MU values were observed in shoots of control plants.

No meaningful variations were detected among the two cultivars (Bluecrop and Duke) when compared with the three different substrates (Table S4). The only variations were detected for Mn indices, in which Bluecrop showed a higher BF in TS0 (+63%) and greater root MU (+29%) in TS50 than Duke.

4. Discussion

4.1. Element Content in Different Plant Organs of Highbush Blueberry

Analyzing plant organs is crucial to estimating nutrient and metal uptake levels and understanding element mobility in highbush blueberries. While such studies on *V. corymbosum* are limited to Mn, extensive research exists on the closely related wild species *V. myrtillus*, renowned for its remarkable metal tolerance. The study by Karlsons et al. [45] is the only one reporting the concentrations of twelve biologically essential elements, including Fe, Mn, Zn, and Cu, in leaf samples of *V. corymbosum*. However, these data are challenging to compare, as the reported values are averages derived exclusively from leaf samples collected at various production sites in Latvia, without any details on cultivation practices or soil conditions.

Our findings showed a consistently higher nutrient concentration in the aboveground biomass of highbush blueberries compared to roots (Table S2). Specifically, P was identified as the most abundant nutrient, followed by K and Ca, with a consistent distribution pattern across all substrates and cultivars. Among major elements, Ca and K were distributed throughout the plant, while Mg accumulated predominantly in leaves. Similar trends were observed in studies on *V. myrtillus* by Parzych [46], Brašanac-Vukanović et al. [18], and Kubov et al. [47], which reported higher nutrient concentrations in leaves and shoots compared to roots. The content of the heavy metals investigated in highbush blueberry shoots followed the decreasing order: $Mn > Fe > Zn > Cu$ (Table S3), in line with results by Parzych [17] for *V. myrtillus*. Considering the normal and toxic concentration levels of heavy metals in plants [48], all metal concentrations remained within normal limits, except

for Mn. Indeed, this study revealed elevated levels of Cr, Fe, Ni, Pb, and Zn in all organs of the two investigated cvs grown in TS-based substrates, with only a few exceptions (Cu in stem, Fe in Bluecrop stem, and Zn in Duke root). However, the concentrations of these metals remained well below the toxicity threshold values for plants [48].

Compared to conventional approaches that evaluate each component individually, the ANE–ATE method provides a more comprehensive interpretation of the mineral supply status of plants, highlighting abnormalities in the relationships between components [36]. This method revealed that in the substrate with the highest ATE sum, the total macronutrient accumulation was significantly lower compared to the other substrates (Tables 6 and 7). Specifically, the macronutrient content in highbush blueberry shoots, as determined by the ANE method, indicated that the control substrate (TS0) was less nutrient-rich than TS50 and TS100, with percentages of Ca and Na in macronutrients being lower in plants grown in TS0. The increase in Ca in highbush blueberry organs may be related to the high Ca concentration in TS50 and TS100 being ~5 times greater than the control. In this context, many studies report the Ca uptake by plants in Ca-rich substrates [49,50]. Moreover, differences in nutrient and metal bioaccumulation between the control and polluted conditions can be most likely attributed to variations in metal mobility and selective root uptake. Thus, it is reasonable to assume that highbush blueberry plants absorbed metals according to their concentration and availability, consistent with previous studies on soil metal mobility and plant accumulation [51–53].

In this context, pH plays a key role in the availability of elements for plants, which can affect plant performance. In the current study, a reduction in plant growth and biomass production was observed in TS-based substrates (Table 4), which showed higher pH levels (6.72 and 7.81) than the control (4.59). *V. corymbosum* typically prefers slightly acidic substrates (4.0–5.0) [54], with pH = 6.5 considered the upper threshold for optimal growth in most cultivars [55]. Our findings align with those of Yang et al. [55], who reported a decline in plant growth parameters and biomass across four different cultivars when grown in substrates with pH > 6.5. We used cultivars (Duke and Bluecrop) that were different than the ones studied by the mentioned authors (Primadonna, Anna, Baldwin, and Bluegold), further confirming a common trend across various blueberry cultivars. In addition, our study revealed a reduction in chlorophyll content (SPAD) in TS-based substrates (Table 5), consistent with the findings of Yang et al. [55], who observed a decrease in chlorophyll levels with increasing pH in the growing medium. In our study, the pH of the control substrate was slightly acidic, a condition that promotes the bioavailability of certain metals, including Mn [53,56]. Soil pH is widely recognized as one of the most influential factors affecting the desorption and bioavailability of heavy metals, as it strongly impacts their solubility and speciation in both the substrate matrix and solution [31,53,57]. At higher pH levels, metals tend to form insoluble mineral phosphates and carbonates, while at lower pH levels, they are more bioavailable as free ionic species or soluble organometallic compounds [58].

Mn concentrations in the stems and roots of *V. corymbosum* were within the toxicity range for plants (400–1000 mg kg⁻¹) [48] in the less polluted control substrate. Notably, the control blueberry stems had higher Mn concentrations than stems from the TS-based substrates, with Bluecrop having 758 mg kg⁻¹ and Duke having 690 mg kg⁻¹. However, while Mn accumulation is known to inhibit plant growth and reduce biomass production and pigment concentrations [28,59–62], no such correlation was observed between the elevated Mn levels and reduced growth in the control plants. These findings agree with the hypothesis that some species of the *Vaccinium* genus, including *V. myrtillus*, can tolerate higher levels of Mn toxicity than other plants [15,63]. For instance, research has established that Ericaceous species, including *V. myrtillus*, accumulate Mn regardless of the soil Mn

concentration [10,14,64]. In addition, several works have proved that *V. myrtillus* is an Mn accumulator and uses high concentrations of this element for its normal physiological functions [14,17,18,20,65,66]. Some studies indicated that Mn accumulation in plants is influenced by input levels, with the highest concentrations generally found in the leaves [19]. However, when comparing our data to those reported by Karlsons et al. [45], Mn levels in *V. corymbosum* leaves were lower. In this regard, the sampling period might play a significant role in the Mn content observed in different plant organs. Once absorbed, Mn remains in leaves due to limited re-mobilization [67] and is primarily transported via transpiration [68,69]. Mature leaves accumulate more Mn than stems, but during senescence, leaf fall removes excess Mn, mitigating toxicity. In contrast, evergreen stems retain Mn year-round, maintaining relatively stable concentrations [70]. In addition, the study by Tamir et al. [50] reports that the Mn translocation restriction to the plant shoots is related to the high Ca concentration in alkaline conditions. Their results showed that blueberries grown in alkaline (pH = 7.5) and Ca-rich conditions exhibited significant reductions in chlorophyll content and biomass, confirming our observation. Moreover, their findings revealed that excess Ca in high-pH conditions accumulates in root epidermal cells, leading to cell-wall thickening and reduced root elongation. This phenomenon further contributes to Mn deficiency in leaves, as Mn uptake and translocation are hindered by excessive Ca binding in root tissues. Thus, the lower Mn accumulation in blueberries grown in TS-based substrates than the control may be due to the high Ca content observed in the substrate.

The concentration of Zn in *V. corymbosum* exhibited only moderate variation across aboveground and underground plant parts and substrates (Table S3). Zn levels generally fell within the range of 15–30 mg·kg⁻¹, which is considered sufficient to meet the physiological needs of most plants, consistent with the findings of Mróz and Demczuk [14], Kozanecka et al. [71], and Pająk and Jasik [72]. The highest Zn concentrations were observed in blueberry shoots, aligning with the observations of Wasilowska and Gworek [73] and Kozanecka et al. [71], who noted that deciduous shrubs store nutrients in stems and roots before leaf fall, resulting in higher nutrient accumulation in these parts compared to leaves.

The distribution of Fe showed the highest concentration in the roots, consistent with the findings of Wasilowska and Gworek [73] (139.6–707.2 mg·kg⁻¹), while lower concentrations were observed in the shoots and leaves. Similarly, Parzych [17] and Kandzióra-Ciupa et al. [74], in a study on *V. myrtillus*, highlighted that underground shoots exhibited the highest Fe concentrations. The Fe levels found in the leaves are consistent with those reported by Karlsons et al. [45] for *V. corymbosum*. Considering stems and leaves together, the values observed in both cultivars across all tested substrates fell within the ranges reported in previous studies for bilberry shoots, such as those by Mróz and Demczuk (120.0–217.0 mg·kg⁻¹) [14] and Gworek and Degórski (95.0–104.0 mg·kg⁻¹) [65].

The Cu content in blueberry plant parts ranged from a minimum of 2.0 mg·kg⁻¹ in Duke stems to 7.7 mg·kg⁻¹ in Bluecrop roots (Table S3). Our values for leaf samples perfectly align with those reported for *V. corymbosum* by Karlsons et al. [45]. On average, Cu levels in aboveground plant parts are typically reported to range from 5 to 15 mg·kg⁻¹ [75]. Copper in plants demonstrates low mobility, with approximately 2 mg·kg⁻¹ being sufficient to meet the physiological needs of most plants. Generally, Cu concentrations in plants are below 4–5 mg·kg⁻¹, although variations depend on factors such as plant part, developmental stage, species, and variety. Accordingly, the Cu content in blueberry plants grown in control and TS-based substrates is relatively low, posing no threat, while remaining adequate to satisfy the physiological requirements of *V. corymbosum*.

4.2. Accumulation Indices in Highbush Blueberry

The ability of plants to extract, translocate, and accumulate heavy metals can be assessed by examining the accumulation indices. Depending on the metal type, substrate, and cultivar, varying values for TC, TF, BF, EF, RE, and MU were observed (Table 8).

The root system serves as the primary gateway for the penetration of trace elements into plants, with the intensity of the process being directly dependent on the concentration and availability of elements in substrates and soils [76,77]. In our study, the average translocation of heavy metals (TC) from soil to roots follows the sequence: Mn (3.61) > Cu (0.20) > Zn (0.19) > Ni (0.06) > Cr, Fe and Pb (0.03). These values were all below 1, except for Mn, indicating that blueberry plants did not accumulate significant amounts of metals in their roots, despite the high levels of Cr, Fe, Ni, Pb, Cu, and Zn present in the initial TS50 and TS100 substrates. Although the initial Mn content was similar in all substrates, TC values confirmed that Mn uptake by the roots was particularly elevated in the control.

TF levels highlighted differences in the mobility and transport mechanisms of metals within plants. These patterns did not follow a consistent trend and varied depending on the specific metal. TF values exceeding 1 were observed for Cu (except in TS50), Mn, Ni, and Zn. The highest Mn and Zn TF values observed in TS100 suggest that the Bluecrop and Duke cultivars efficiently translocate these metals from roots to shoots, highlighting their potential as accumulators and their ability to absorb these metals when grown in metal-rich substrates. Previous studies [10,14,64,74,78] have similarly shown that closely related species, such as *V. myrtillus*, efficiently accumulate Mn and Zn, reporting TF values consistent with our findings [18]. In contrast, the relatively low TF values for Fe and Pb likely reflect their limited mobility, as these metals primarily accumulated in the roots. Chromium, on the other hand, exhibited a TF > 1 only in the control samples, which may be attributed to the sediment's more refractory matrix and typical reducing conditions, as well as the enhanced Cr mobility in the acidic peat substrate.

BF is a valuable indicator of a plant's ability to accumulate specific metals from the environment [39]. Our results show elevated BF levels for Mn across all plant organs, with the highest values observed in plants grown in TS0 and lower values in TS100. This trend may be related to the competition of Ca, which disrupts the root system, reducing Mn uptake [50]. Additionally, BF values were higher in Bluecrop compared to Duke (Table S4). Plant growth inhibition has been reported in *V. corymbosum* plants exposed to Mn²⁺ toxicity [29,79]. However, studies on various blueberry cultivars grown in pots under greenhouse conditions and subjected to Mn toxicity (1000 µM) have revealed that Mn accumulation in shoot tissues varies by cultivar, allowing them to be categorized as either Mn-sensitive or Mn-tolerant [29]. Notably, the Duke cultivar has been identified as Mn-resistant, demonstrating distinct mechanisms of Mn resistance. Although there are no specific studies on the Bluecrop cv, the observed biomass and ecophysiological parameters, along with the absence of clear toxicity symptoms and the higher BF values, suggest that Bluecrop could likely be considered among the Mn-tolerant highbush blueberry cultivars. Our results indicated BF values for Zn accumulation lower than 1 but close to 1 in plants grown on the control substrate. These values are lower than those reported by Brašanac-Vukanović et al. [18] for bilberry grown in uncontaminated environments in Montenegro. Copper was evenly distributed across all parts of the blueberry, with BF values ranging from 0.09 to 0.86. These findings are consistent with earlier studies from northern Europe, which observed similar levels of Cu accumulation in bilberry [10,18]. These findings suggest that in TS-based substrates, the presence of antagonistic metal pairs like Cu–Zn [80,81] might have affected Cu uptake by highbush blueberry plants. Our results also showed that Cr and Pb were present in relatively low amounts across all parts of the plant, with

BF values ranging from 0.01 to 0.1, indicating that highbush blueberry did not accumulate these metals. The plant's absorption of Cr and Pb, as well as their translocation from root to shoot, may be inhibited by the presence of Cu, Zn, Fe, and Ni [39].

The highest EFs in highbush blueberries were recorded for Pb, consistent with the findings reported by Kandziora-Ciupa et al. [20] for bilberry. Cr EF values > 1.5 were also observed, indicating a moderate accumulation potential. The TCs from soil to root and the TFs from root to aerial parts were lower than the EFs, and their trends did not align. This suggests that despite high heavy metal concentrations in polluted substrates, their mobility and uptake by different plant organs are somewhat restricted. A similar observation was made by Singh et al. [82], who studied the accumulation and translocation of heavy metals in plants grown in contaminated soil. Contributing factors such as pH, cation exchange capacity (CEC), and organic matter content play a significant role in metal bioavailability, as well as plant-specific mechanisms (e.g., metal sequestration in root cell walls and phytochelation), which further modulate translocation [63,83].

The RE, a measure of heavy metal extraction, exceeded 90% for Cu in TS100 and was over 80% for Mn in TS0. These high values may depend on the enhanced bioavailability of Cu and Mn under the specific substrate mixture, probably affected by the previously mentioned substrate properties such as pH that can alter the chemical activity of metals by breaking bonds between metal ions and organic and inorganic compounds in soils [84].

The decrease in Mn content in the substrates was accompanied by particularly high Mn MU values in *V. corymbosum* roots and shoots grown in TS0 (Tables 8 and S4). These high values are particularly significant when paired with high biomass, as observed in the control plants, suggesting potential for Mn bioextraction. Conversely, the lower MU values for Cu observed in TS100 are likely correlated with the reduced biomass of plants grown in this substrate, which was over one-third lower than in the control.

5. Conclusions

Our study found that metal accumulation efficiency in plant tissues varied significantly depending on the element, cultivar, and organ. The results of this study indicate that blueberries retained Mn and Zn in stems and roots, with lower accumulation in the leaves. Cu, Fe, and Pb, were predominantly accumulated in the roots. Both cultivars acted as excluders for these metals, limiting their transport from roots to shoots. Conversely, for Mn, the transfer coefficient (TC), the translocation factor (TF), and the BF bioaccumulation factor (BF) were mostly greater than 1, highlighting its pronounced capacity for manganese accumulation. Specifically, BF values were found to occur in the following ascending order: Fe and Pb < Cr < Cu < Ni < Zn < Mn, indicating that blueberry accumulated Mn to a greater extent compared to other metals, followed by Zn. On an individual metal basis, Mn and Zn were found to accumulate more in the Bluecrop cultivar when grown on an unpolluted control substrate. This study highlights the ability of highbush blueberry plants to selectively accumulate heavy metals when grown in polluted substrates under suitable conditions, making them a valuable model for understanding metal accumulation mechanisms in the Ericaceae family.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15020503/s1>, Table S1: Effect of substrate composition (Su) and cultivar (Cu) on macronutrient and heavy metal concentrations of substrate samples at the end of the experiment (tf); Table S2: Macroelement concentration (mg kg⁻¹ DM) in the different plant parts of *V. corymbosum* cvs Bluecrop and Duke; Table S3: Trace element concentration (mg kg⁻¹ DM) in the different plant parts of *V. corymbosum* cvs Bluecrop and Duke; Table S4: Accumulation indices in *V. corymbosum* cvs Bluecrop and Duke.

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