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Switchgrass and Giant Reed Energy Potential when Cultivated in Heavy Metals Contaminated Soils

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Abstract: The cultivation of energy crops on degraded soils contributes to reduce the risks associated with land use change, and the biomass may represent an additional revenue as a feedstock for bioenergy. Switchgrass and giant reed were tested under 300 and 600 mg Cr kg^{-1}, 110 and 220 mg Ni kg^{-1}, and 4 and 8 mg Cd kg^{-1} contaminated soils, in a two year pot experiment. Switchgrass yields (average aerial 330 g.m^{-2} and below ground 430 g.m^{-2}), after the second year harvest, were not affected by Cd contamination and 110 mg Ni kg^{-1}, but 220 mg Ni kg^{-1} significantly affected the yields (55–60% reduction). A total plant loss was observed in Cr-contaminated pots. Giant reed aboveground yields (control: 410 g.m^{-2}), in the second year harvest, were significantly affected by all metals and levels of contamination (30–70% reduction), except in 110 mg Ni kg^{-1} pots. The belowground biomass yields (average 1600 g.m^{-2}) were not affected by the tested metals. Contamination did not affect the high heating value (HHV) of switchgrass (average 18.4 MJ.kg^{-1}) and giant reed aerial fractions (average 18.9 MJ.kg^{-1}, stems, and 18.1 MJ.kg^{-1}, leaves), harvested in the second year, indicating that the biomass can be exploited for bioenergy.

Keywords: *Arundo donax*; *Panicum virgatum*; heavy metals; phytoremediation; contaminated soils; low ILUC crops

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1. Introduction

Petroleum is currently present in every aspect of our lives, either in fuels, clothes, computer components, or cosmetics. The development of alternative feedstocks to substitute petroleum is a challenge. We may have solar photovoltaic, hydropower, and wind power plants for energy production, but an alternative that is suitable for replacing petroleum in its many uses is biomass, through the conversion of refineries into biorefineries [1,2].

Biomass production and utilization in industrial processes is a growing practice that represents a renewable and more sustainable feedstock when compared with petroleum [3]. The versatility of biomass allows the production of fuels in different states: solid, liquid, or gaseous. In addition, the production of fibers and chemicals can replace petroleum with minor adaptations in industries or refineries, becoming an excellent alternative to change to a more sustainable feedstock [4–10]. Moreover, utilizing biomass in the energy sector can reduce greenhouse gas emissions, helping the European Union (EU) to achieve its goal of no net emissions by 2050, a compromise firmed in the European Green Deal [11]. Although the advantages of using biomass in the different industries comprise an attractive choice, the energetic conversion of biomass could be harmed by the Indirect Land Use Change (ILUC) effects [11,12]. The growing of non-food crops increases the demand for soil, which can cause a cascade effect, elevating the cost of food production and consequently increasing its price [13,14]. To avoid a scenario that can threaten the food supply in EU, especially low-income families, lands unsuitable for food production are in fact an opportunity for the cultivation of non-food crops [15–17]. Soils contaminated with heavy metals are examples of unsuitable soils that can be used to cultivate dedicated crops, as experienced in Germany with industrial hemp [18], in Italy with giant reed [19], or in USA with switchgrass [20].

The cultivation of biomass depends on factors that must be considered, such as climate conditions, cultivation methods, soil, and water quality [21]. Regarding soil and water quality, the contamination of these resources constitutes a growing problem and represents a challenge for industrial crops cultivation. Yields and biomass quality can be affected, generating contaminated residues, damaging equipment, or retarding processes like fermentation [22–24]. Moreover, the accumulation of heavy metals in the biomass requires special attention once this can lead to its accumulation in the food chain. Despite some heavy metals acting as micronutrients in some organisms, they can cause severe health issues to humans [25,26]. However, although contaminated soils may affect yields and biomass quality [27], industrial crops cultivated in soils containing high concentrations of heavy metals can play an essential role from an environmental point of view. Besides being a renewable raw material, industrial crops can tackle the problem of soil contamination, accumulating or stabilizing the heavy metals in a phytoremediation technique while producing feedstock for industrial processes [28–30].

In this work, two energy crops were selected to be tested in soils contaminated with nickel (Ni), cadmium (Cd), and chromium (Cr)–*Panicum virgatum* L. (switchgrass) and *Arundo donax* L. (giant reed). Both crops have the potential to produce high amounts of biomass, tolerating hostile environments such as water stress, adverse climate conditions, and soil contamination, as reported for these and other perennial grasses [31–37], making them a possible feedstock for biorefinery processes. The contaminants were chosen based on the different urban, industrial, and agricultural processes that generate this heavy metal contamination. Nickel, for example, is a metal used in metallurgic, food processing, and chemical industries, Cr derives from mining and refining processes, and Cd comes from burning fossil fuels, or the agricultural application of fertilizers and sewage sludges [38,39]. Giant reed has already been tested in Ni-, Cd-, and Cr-contaminated soils [40–44], demonstrating a high tolerance and accumulation potential for these heavy metals. Switchgrass was also tested in Cd- and Cr- contaminated soils [45,46], with promising potential for phytoremediation. However, the experimental design from those experiments was different from the one proposed in the current study. In the previous studies with these two crops in Cr-, Ni-, and Cd-contaminated soils, tests were made with different concentrations and different sources of contamination. In addition, the focus in these studies was mainly on the phytoremediation action and use of chelating agents or arbuscular mycorrhiza to improve the tolerance, and phytoextraction was also a target. Moreover, for most of the studies, only one year of experiment was reported, or the plants were only in contact with the contamination for a finite number of days. Very few studies address how the characteristics of the biomass (e.g., ash content, nitrogen content, heating value) for bioenergy are affected by the soil contamination, nor how these crops behave after more than one growing season in contaminated soils, making it challenging to provide information on the influence of a particular metal on the plant's growth and biomass characteristics over several growing seasons.

Therefore, the current study aims to increase knowledge as to how the cultivation of these two energy crops–giant reed and switchgrass–in soils polluted with Ni, Cr, or Cd can exert a phytoremediation action, merged with its possible exploitation for bioenergy, thus contributing to a resource-efficient bioeconomy. In this sense, the study was planned to be carried out for more than one year; this will provide more information as to how these two perennial crops behave in a contaminated soil along several growing seasons. In fact, these crops reach maturity only after the second harvest year, once they take the first growing season to develop their root system and rhizomes [35]. Consequently, extending the study to a second year will provide more reliable information on the effect of contamination on biomass productivity and quality, an information that few studies have addressed so far (some studies made with giant reed were performed along two years [40,42–44], but with switchgrass, the studies were performed for a short period–40 days [46] or 20 weeks [45]). In addition, this study will evaluate the quality of the harvested biomass, e.g., ash content, nitrogen content, calorific value, providing data that will allow a better assessment of the value of this biomass for energy–something that is also poorly evidenced in existing studies (and with these two crops, no such studies have ever been conducted). The contribution of the data presented in this work will help to identify the challenges and the opportunities of the production of clean bioenergy through the use of lignocellulosic non-food energy crops (listed in the Annex IX of the RED II recast [47]) cultivated on contaminated land and how these biomasses can help to cover the global energy demand expected until 2050 [48].

2. Materials and Methods

2.1. Experimental Setup

The experiment was conducted in the *Campus* area of the NOVA School of Sciences and Technology, Universidade NOVA de Lisboa. University of Bologna provided switchgrass seeds, and rhizomes of giant reed were collected in the *Campus* area. The experiment was conducted in pots, during two years. Each pot was filled with 12 kg of sieved soil (10 mm) also collected in the *Campus* (June 2018, control soil). The pots had an area of 0.07069 m^2.

The soil was artificially contaminated by mixing contaminated sludges and salt solutions with the control soil in order to ensure a high bioavailability of the metals to the plants. Nickel contamination was performed by mixing an industrial sludge containing 36% Ni (dry weight basis), supplied by Centro para a Valorização de Resíduos, CVR, an association dedicated to provide solutions to wastes recovery, and a salt solution of nickel sulfate ($NiSO_4.6H_2O$). Cadmium trials were contaminated with a solid residue rich in Cd (14%, dry weight basis), obtained through a recovery process from Ni-Cd batteries (supplied by Instituto Politécnico de Portalegre, IPP), mixed with a salt solution of cadmium nitrate ($Cd(NO_3)_2.4H_2O$). Chromium contamination was performed by mixing chromium (III) chloride ($CrCl_3.6H_2O$) and potassium dichromate (K_2CrO_4) (equimolar quantities) with an industrial sludge from AUSTRA-CTIC association, located in Alcanena, with Cr (8 $g.kg^{-1}$, dry weight basis), and other metals. After soil preparation, giant reed pots received two rhizomes (10 cm deep) [40], and switchgrass pots were seeded. Fertilization was applied in the switchgrass and giant reed pots: 3 g N m^{-2} (nitrolusal, mixture of NH_4NO_3 + $CaCO_3$, 27% N), 3 g N m^{-2} (urea, 46% N), 23 g P_2O_5 m^{-2} (superphosphate, 18% P_2O_5), and 17 g K_2O m^{-2} (potassium sulfate, 51% K_2O). The urea fertilization was made when the height of the plants reached 30–40 cm. In the second year, the same NK fertilization was carried out when plants presented 30–40 cm height. As the P fertilizer applied in the first year is sufficient for the nutrition of these crops for at least 10 years [35], no more P was added in the second year. For each heavy metal, two different concentrations were essayed to study the sole effect of each contaminant: (a) Ni 110 and 220 $mg.kg^{-1}$ dry matter, Ni_{110}, and Ni_{220}, respectively; (b) Cd 4 and 8 $mg.kg^{-1}$ dry matter, Cd_4 and Cd_8, respectively; (c) Cr 300 and 600 $mg.kg^{-1}$ dry matter, Cr_{300}, and Cr_{600}, respectively. The two concentrations tested (low and high) match, for each element, the limit value and twice this value in soil according to the Portuguese Decree-Law [49]. These limits are associated to the total concentration of the respective heavy metal in the soil. Full irrigation was provided to all the pots to surpass water stress (950 mm), and each species/ type and level of heavy metal contamination was evaluated in triplicate. In January 2019 and January 2020, when each growing season was reaching its end, the plants were harvested and the above ground biomass production ($g.m^{-2}$) was monitored. The belowground productivity and the biomass characterization (e.g., ash, nitrogen, and heavy metal content) was also evaluated in the second harvest. Percolated waters were collected and analyzed just before the second harvest.

2.2. Soil Analyses

At the start of the trials, the top 30 cm soil was collected in the Campus. The soil was sieved through a 2 mm mesh after being dried in an oven at a temperature of 30 ± 5 °C [50–52], and further analysed for electrical conductivity, pH, total organic matter, cation exchange capacity (CEC), total N and P, extractable phosphates, and total metal content (Na, K, Ca, Mg, Cd, Cr, Ni). A conductivity meter MC226 Mettler Toledo and a pH-meter micropH2001 Crison were utilized to measure the electrical conductivity and the soil pH (in H_2O) [51]. The Chapman method [52] and the Walkley-Black method [53] were used to measure the CEC and soil organic matter, respectively. Total nitrogen and total phosphorous content were measured following the Kjeldahl method and the spectrophotometric method on the digested samples [50]. Extractable phosphates were quantified by spectrophotometry following extraction of the soil with 0.5 M $NaHCO_3$ [54,55]. A digestion with aqua regia was made in the soils and total metal content was quantified, in the digested samples, by atomic absorption spectrometry (ZEEnit 700, Analytic Jena, Germany) in agreement with ISO 11466 [56]. Soils were also analyzed after the artificial contamination with Cd, Cr, and Ni for the total [56] and the bioavailable [57] content of each metal by atomic absorption spectroscopy. The bioavailable fraction of Cd, Cr, and Ni in the soils was quantified in the extracts obtained from the soils with 0.05 M EDTA at pH 7.5 [57].

2.3. Plant Analyses

After each harvest, giant reed and switchgrass aboveground biomass was collected. In the case of giant reed, aerial biomass was separated by stems and leaves. The belowground biomass (roots and rhizomes) were also collected from the pots after the second harvest. The plant biomass weight of each fraction, collected and dried at 70 °C, allowed to assess the belowground and aerial productivity ($g.m^{-2}$, dry basis) of both biomasses. Before analysis, the plant fractions were ground and stored. Harvested material on the second growing cycle end was characterized and results expressed to the biomass dried in an oven at 105 °C. Nitrogen and phosphorous analysis was made in the digested samples following the Kjeldahl method and the spectrophotometric method [50], respectively. The determination of ash in the different biomass fractions, expressed as the residual mass left after oxidation at 575 ± 25 °C, in a muffle (L3/11/C6, Nabertherm, Lilienthal, Germany), followed the ASTM E1755 test [58]. The metal concentration (Ni, Cd, or Cr, depending on the source of contamination), and Na, K, Mg, Ca, Fe, and Mn contents were quantified by atomic absorption spectrometry in the nitric acid dissolved ash residues [59]. The procedure to determine the volatile material was made according to the U.S. Bureau of Mines by the difference in sample weight before and after the calcination process at 900 ± 20 °C in a muffle [60]. The difference in the weight is related to the volatile material degraded during the calcination. The fixed carbon was determined by subtracting the total dry biomass's volatile material and ash percentual. Then, the high heating value (HHV) expressed in $MJ.kg^{-1}$ dry weight (dw) was calculated, taking into consideration both the volatile matter (VM) and the fixed carbon (FC), as given in Equation (1) [61]:

$$\text{HHV (MJ.kg}^{-1}\text{, dw)} = 0.1905 \times [\text{VM, \% w/w dw}] + 0.2521 \times [\text{FC, \% w/w dw}] \quad (1)$$

Hemicellulose, cellulose, and lignin were determined, following a sequential extraction with neutral and acid detergents, a treatment with 72% sulfuric acid and, finally, an incineration at 550 °C of the final residue [62].

2.4. Percolated Waters Analyses

Percolated waters were collected just before the second harvest, and their contaminant content (Cd, Cr, or Ni) was determined on acidified and filtered samples by atomic absorption spectrometry.

2.5. Mathematical Formulas

To analyze the fouling/slagging propensity of the biomass ashes given the ash composition, an alkali index can be calculated based on the composition of the biomasses. The sum of sodium and potassium oxides, which melt at low temperature ($Na_2O + K_2O$), expressed in kg/GJ, can be used to calculate the fouling and the slagging probability to occur (Equation (2)) [63]:

$$\mathrm{AI} = (\mathrm{Na_2O} + \mathrm{K_2O})\ \mathrm{kg/GJ} \tag{2}$$

Different parameters can be calculated using the biomass characterization to determine the plants' phytoremediation potential.

The tolerance index (TI) evaluates the crop tolerance to the heavy metal contamination (Equation (3)) [64,65]:

$$\mathrm{TI} = \frac{\text{dry biomass weight of contaminated plants; g.m}^{-2}}{\text{dry biomass weight of control plants; g.m}^{-2}} \tag{3}$$

The modified accumulation index (mAI) will be used to determine the plant's capability to uptake and accumulate a contaminant in more significant amounts than it usually does (Equation (4)) [40]:

$$\mathrm{mAI} = \frac{\text{metal accumulation in the contaminated plants; mg.m}^{-2}}{\text{metal accumulation in control plants; mg.m}^{-2}} \tag{4}$$

The modified bioconcentration factor (mBCF) evaluates the heavy metal concentration in the different fractions of the biomass compared with the metal concentration bioavailable to plants (Equation (5)) [40]:

$$\mathrm{mBCF} = \frac{\text{metal concentration in the plant fraction; mg.kg}^{-1}}{\text{bioavaiable metal concentration in the soil; mg.kg}^{-1}} \tag{5}$$

Instead of using the total metal concentration of the soil, the use of the bioavailable content (to the plants) in the soil represents the ability of the plants to extract and concentrate the metals, helping to decontaminate the soil more realistically [40,66].

The modified bioaccumulation factor (mBAF) is calculated to assess the plants' capability to take away the contaminants from the soil (Equation (6)) [40]:

$$\mathrm{mBAF}(\%) = \frac{\text{metal accumulation in the plant fraction; mg.m}^{-2}}{\text{bioavaiable metal content in the soil; mg.m}^{-2}} \times 100 \tag{6}$$

The transfer of metals from the belowground biomass to the harvestable aerial fractions, measured through the translocation factor (TF) and the modified translocation factor (mTF) (Equation (7) and Equation (8), respectively), can be used to determine the potential application of the crops in phytoextraction treatments [40]:

$$\mathrm{TF} = \frac{\text{metal concentration in the aboveground plant fraction; mg.kg}^{-1}}{\text{metal concentration in the belowground plant fraction; mg.kg}^{-1}} \tag{7}$$

$$\mathrm{mTF} = \frac{\text{metal accumulation in the aboveground plant fraction; mg.m}^{-2}}{\text{metal accumulation in the belowground plant fraction; mg.m}^{-2}} \tag{8}$$

The calculation of mAI and mTF combines the biomass metal concentration in each fraction with the biomass production per area. When the following indexes are greater than one (>1), mAIs, mBCFs, TFs, and mTFs, the plants show phytoextraction potential [40].

2.6. Statistical Analyses

Results obtained in this study were statistically interpreted using one-way ANOVA (analysis of variance) followed by the Tuckey test to find means that are significantly different from each other (IBM SPSS Statistics version 23, IBM, Armonk, NY, USA). The results, obtained from triplicate analysis, were presented as the mean ± standard deviation. The propagation of the deviation obtained in contaminated plants and control plants was used to calculate the uncertainty of the TI and mAI results.

3. Results and Discussion

3.1. Soil Characterization

The uncontaminated soil's characterization can be seen in Table 1. The soil presents low levels of organic matter, N and P. In addition, it presents an alkaline pH with low initial levels of the studied contaminants, Cd, Ni, and Cr. Due to soil's high pH, the high CEC value may indicate a likelihood of overestimating exchangeable bases by extracting nonexchangeable Ca and Mg from carbonate solids [52].

Table 1. Control soil physical and chemical characteristics.

Parameters	
pH	8.4 ± 0.2
Electrical conductivity (dS m^{-1})	0.29 ± 0.03
CEC (cmol(+)kg^{-1}, dw)	40 ± 3
Total organic carbon (g C kg^{-1}, dw)	8.6 ± 0.8
Total nitrogen (g N kg^{-1}, dw)	1.0 ± 0.2
Total phosphorus (g P kg^{-1}, dw)	0.72 ± 0.01
Available phosphorus (mg P kg^{-1}, DW)	216 ± 10
Total potassium (g K kg^{-1}, dw)	2.15 ± 0.01
Total calcium (g Ca kg^{-1}, dw)	20 ± 6
Total sodium (g Na kg^{-1}, dw)	8 ± 2
Total magnesium (g Mg kg^{-1}, dw)	5.30 ± 0.08
Total cadmium (mg Cd kg^{-1}, dw)	1.0 ± 0.4
Total chromium (mg Cr kg^{-1}, dw)	21 ± 2
Total nickel (mg Ni kg^{-1}, dw)	18 ± 4

dw: dry weight.

The heavy metals' levels of the artificially contaminated soils, built from these initial properties, are presented in Table 2, showing Cd, Cr, and Ni content (total and bioavailable) in the control and artificial soils. The contaminants' bioavailable amount was determined in the extracts obtained with 0.05 M EDTA [57]. Results attained at the start of the experiment show that the bioavailable fractions of Cd, Cr, and Ni, in the artificially contaminated soils, were, respectively, 88–100%, 19–22%, and 75–76% of the total element content. These percentages, in both low and high contamination levels, reflect the quantity of contaminants available to be up taken by the belowground fraction of the plants. Cadmium and Ni showed a higher ratio of bioavailable/total content, indicating that these two metals are more easily mobilized from the soils than Cr, in line with what was reported by Kabata-Pendias [67].

Table 2. Control and artificial soils heavy metals composition.

Main Element of Contamination	Parameters	Soil Type		
		Control	Low	High
Cd	Total cadmium (mg Cd kg^{-1}, dw)	1.0 ± 0.4	4.8 ± 0.9	9.2 ± 1.4
	Bioavailable cadmium (mg Cd kg^{-1}, dw)	0.76 ± 0.05	4.2 ± 0.5	9.9 ± 0.6
Cr	Total chromium (mg Cr kg^{-1}, dw)	21 ± 2	345 ± 56	663 ± 82
	Bioavailable chromium (mg Cr kg^{-1}, dw)	1.308 ± 0.002	67 ± 2	146 ± 13
Ni	Total nickel (mg Ni kg^{-1}, dw)	18 ± 4	118 ± 18	242 ± 32
	Bioavailable nickel (mg Ni kg^{-1}, dw)	5.8 ± 0.4	90 ± 6	182 ± 3

dw: dry weight; Low and high indicates the lower and the higher contamination levels tested in this experiment.

3.2. Effects of Cadmium, Chromium, and Nickel on the Biomass Productivity of Giant Read and Switchgrass

The yield performance of switchgrass grown in Cd and Ni contaminated soils, in two consecutive years, is represented in Figure 1. Results showed that the productivity of switchgrass biomass increased from the first to the second growing cycle. This increase can be explained by the development of the root system that needs to expand in the first growing cycle, reducing the energy of the plant to produce aerial biomass [35]. Regarding the contaminated trials, the biomass productivity was inversely proportional to the concentration of heavy metals. This decrease was noticed for both Cd and Ni contamination levels in the aerial biomass in the first year. In the second harvest, the yields in Cd and Ni contaminated soils with low level of metal contamination were not significantly affected, but the higher contamination levels caused a reduction in biomass productivity, either in the aerial and in the belowground biomass, and this reduction was significant in the Ni_{220} soils.

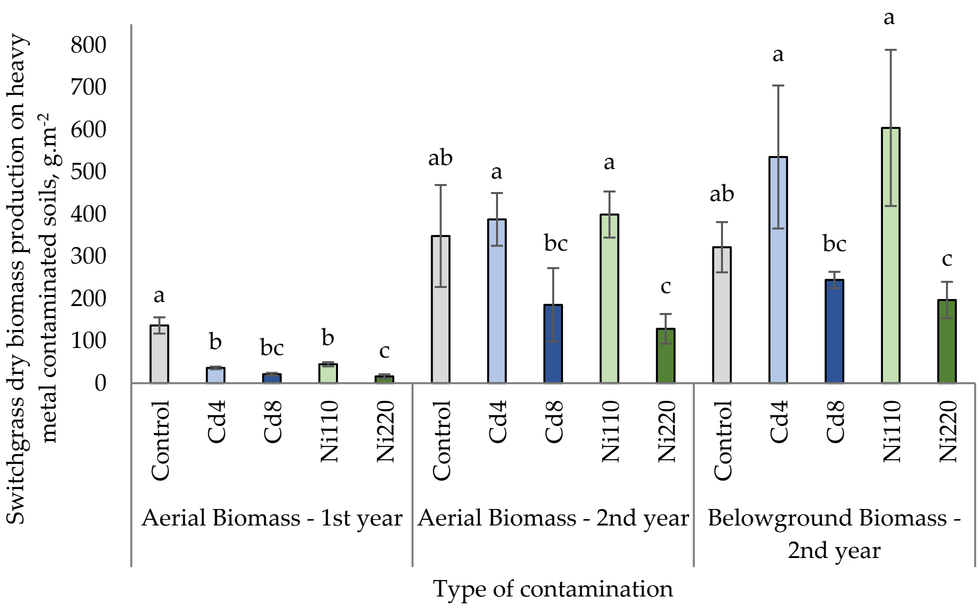

Figure 1. Switchgrass dry biomass production on Cd- and Ni- contaminated soils. For each biomass fraction and year, different lower-case letters indicate statistical significance ($p < 0.05$) between treatments. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

In contrast to the behavior of switchgrass in Ni- and Cd- contaminated soils, Cr contamination, either the low or the high concentration (Cr_{300}, Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter), inhibited the growth of switchgrass. In the first year of the experiments, the seeds germinated but the toxicity of the soils did not allow the development of the seedlings. In the second year, sowing was repeated, and the same problem occurred again with the seedlings.

Figure 2 shows the aerial productivity of giant reed in Cd-, Ni-, and Cr-contaminated soils, in the first two growing cycles. In the first year, the yield was not affected by the heavy metals at their lowest concentration except in the case of chromium. As the concentration of Cr, Cr, and Ni increases, a significant decrease in biomass yield was noticed. Lowest yields were obtained in Ni_{220} and Cr_{600} soils. The yield of control and Ni contamination trials in the second year did not significantly differ from the yield in the first year. Contrarily, the productivity in Cd- and Cr-contaminated soils lowered from the first to the second year, for both levels of contamination. This behavior was opposite to what was seen by Fernando and collaborators in Cr-, Pb-, and Zn-contaminated soils [68]. In the mentioned study, from the first to the second year, the yields increased, on average, by 150%. This pattern is typical from perennial crops, showing that higher energy is used by the plant in the first year to establish its belowground biomass [33,35], as it was seen with switchgrass. In the second harvest, all the metals–Cd, Cr and Ni–caused a reduction in the yields, which was only not significant in the case of the low contamination level of Ni. Like the first year results, as the concentration of Cd and Ni increases, a significant decrease in biomass yield was obtained. In the case of Cr contaminated soils, the yields between low and high level were similar. For the second harvest, lowest yields were observed in Cd_8, Ni_{220}, Cr_{300} and Cr_{600} soils.

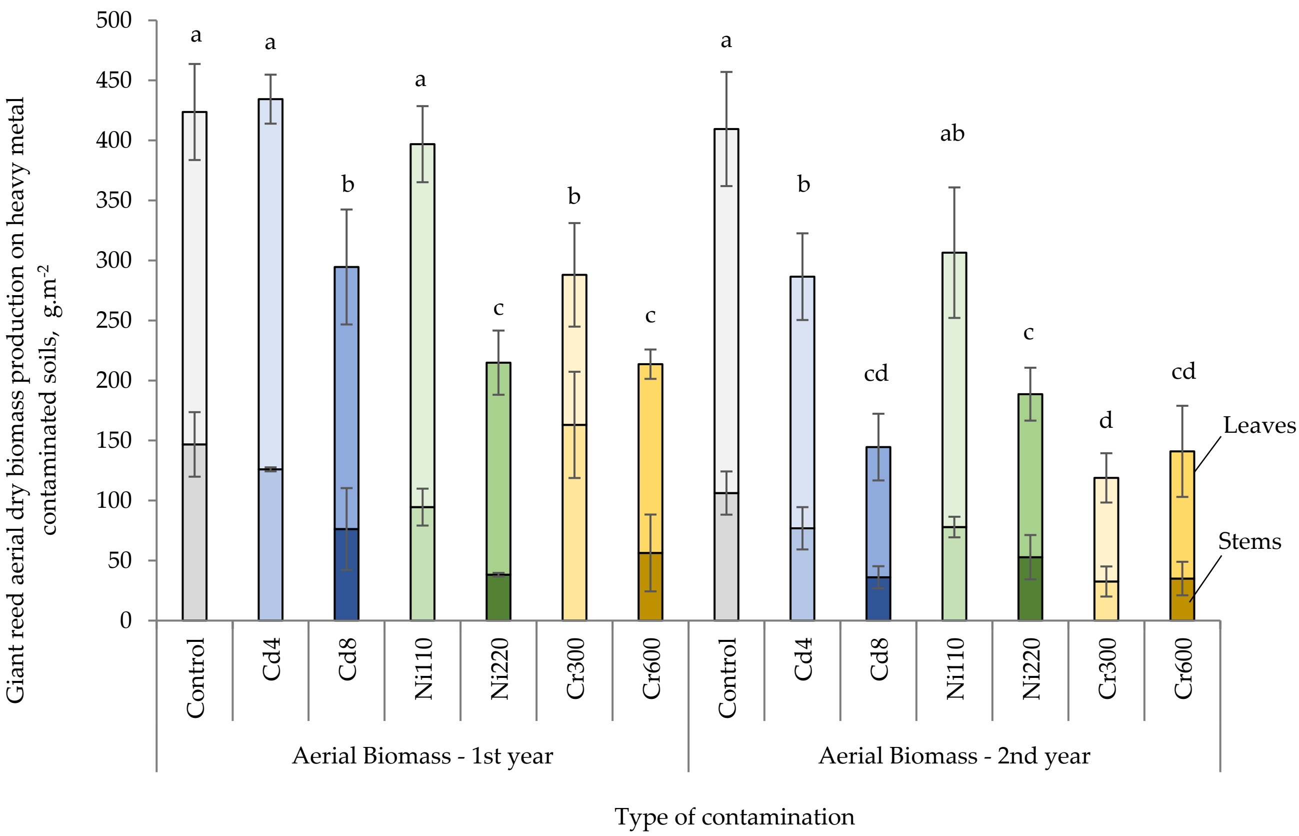

Figure 2. Giant reed aboveground dry biomass production on Cd-, Ni-, and Cr-contaminated soils. For each total aerial biomass (leaves and stems) and year, different lower-case letters indicate statistical significance ($p < 0.05$) between treatments. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter.

The belowground productivity of giant reed is presented in Figure 3. Results show that the contamination did not interfere with belowground biomass productivity, although some reduction was observed in Cd_8 (significant), Ni_{220}, and Cr_{300} soils (not significant).

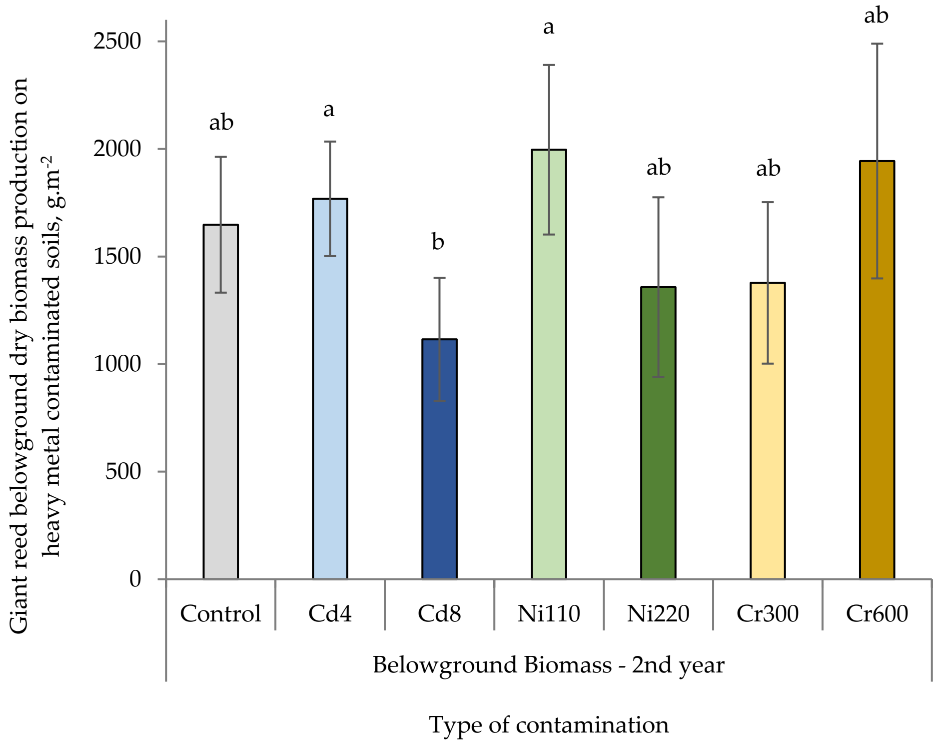

Figure 3. Giant reed belowground dry biomass production on Cd-, Ni-, and Cr-contaminated soils. For each belowground biomass, different lower-case letters indicate statistical significance ($p < 0.05$) between treatments. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter.

A comparison between giant reed yields and switchgrass yields, for the same type of soils, showed that both crops, in terms of aerial biomass, presented similar results in control, Cd-, and Ni-contaminated soils ($p > 0.05$). However, in Cr-contaminated soils, seeds of switchgrass did not germinate and no aerial biomass was made viable, in contrast with the development of giant reed ($p < 0.05$). When comparing the belowground biomass production, it was observed that giant reed presented always significantly higher yields to switchgrass for control and Cd-, Cr-, and Ni-contaminated pots at both contamination levels.

The tolerance index (TI) indicates the influence of the contaminant on the plant's growth, and data obtained for switchgrass and giant reed (second year of experiments) are depicted in Table 3.

Table 3. Tolerance index (TI) of switchgrass and giant reed under Cd-, Cr-, and Ni-contaminated soils.

Contamination	Switchgrass TI		Giant Reed TI	
	Aboveground	Belowground	Aboveground	Belowground
Cd_4	1.1 ± 0.4	1.7 ± 0.6	0.7 ± 0.1	1.1 ± 0.3
Cd_8	0.5 ± 0.3	0.8 ± 0.2	0.4 ± 0.1	0.7 ± 0.2
Cr_{300}	-	-	0.3 ± 0.1	0.8 ± 0.3
Cr_{600}	-	-	0.3 ± 0.1	1.2 ± 0.4
Ni_{110}	1.1 ± 0.4	1.9 ± 0.7	0.8 ± 0.2	1.2 ± 0.3
Ni_{220}	0.4 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.8 ± 0.3

Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter.

Results of TI can help to classify the degree of tolerance of a plant to a metal/type of contamination. The TI can be organized in levels, such as the following ones, high tolerance, TI > 0.75; moderate tolerance: 0.50 < TI < 0.75; low tolerance: 0.25 < TI < 0.50; critical tolerance: TI < 0.25.

Concerning switchgrass, results indicate that, for Ni- and Cd-contaminated soils, only the exposure of the plant to the higher contamination levels resulted in a reduction in biomass production. This reduction was in the range of 50–60% in the aboveground fraction and in the range of 20–40% in the belowground fraction, and was higher (but not significantly) in Ni_{220}-contaminated pots. Therefore, for the low contamination levels, switchgrass showed high tolerance to Cd and Ni; for the high contamination level, switchgrass showed low tolerance to Ni and moderate tolerance to Cd, regarding the production of aboveground biomass, and moderate tolerance to Ni and high tolerance to Cd in terms of the belowground biomass production. Concerning the effect of Cr contamination, results indicate that the crop presented a critical tolerance to this metal once no development of the seedlings was observed.

Arora and collaborators [45] also observed that higher levels of Cd in the soil negatively affected switchgrass productivity. Studying concentrations of 2 to 10 mg.kg^{-1} of Cd contamination in pots for 20 weeks, it was observed that the rise in Cd concentration reduces switchgrass' yield (almost 65% reduction at the highest contamination level). The work of Arora et al. also showed that plant-associated microbes, such as Azospirillum and arbuscular mycorrhizae fungi, can increase the tolerance of switchgrass when it is exposed to Cd contamination [45]. Chen et al. [69] used a higher range of Cd concentrations (0-60 mg.kg^{-1}), leading to a drastic reduction in grass productivity (63% losses at the higher contamination level). In this study, the analysis of the soil's fractions indicated that despite the Cd concentration in soil being high, the fraction mobilized was low, and therefore the plant's survival was possible at 60 mg.kg^{-1}. The toxicity of Cd to switchgrass was also evaluated in a hydroponic experiment using different concentrations of Cd in solution [70]. In the study, although the seeds' germination occurred without showing any impact from the contamination, the plants' development was better in Cd concentrations lower than 20 mg.L^{-1}. To date, experiments of switchgrass in Ni-contaminated soils were not found, but the behavior was similar to what was observed with Cd, although the patterns of toxicity of Cd and Ni in the plants are different [67]. The total loss observed in the Cr-contaminated pots can be justified by the partial contamination with Cr (VI) [71]. Indeed, regarding the oxidation state of the metal, the hexavalent Cr has a higher mobility in the soil, affecting the biomass growth and justifying the losses observed. Li et al. [46] also tested a similar range of Cr contamination (131 to 600 mg.kg^{-1}) using Cr (VI) during three months. Results obtained showed that despite the development of switchgrass in the lower contamination levels, when the concentration was 600 mg.kg^{-1}, aerial biomass productivity losses were around 70%. Belowground biomass also suffered a reduction of around 50%. However, no information was added into the study as to the amount of bioavailable Cr so that a better comparison could be carried out with the results presented in Table 3. Moreover, the higher organic matter of the soil (7.39%) can also explain the higher tolerance of switchgrass to Cr reported by Li and collaborators [46] in their work, compared to the critical tolerance presented in Table 3, once organic matter can trap the Cr in the soil and reduce its toxicity [72].

Concerning giant reed, in terms of the aboveground biomass production, results indicate that, for low contamination levels, the plant showed a high tolerance to Ni, moderate tolerance to Cd, and low tolerance to Cr, and for the high contamination level showed a low tolerance to Cd and Cr and moderate tolerance to Ni. In terms of giant reed belowground biomass, the plant exhibited high tolerance to all the metals and levels, except to the high level of Cd, where the tolerance exhibited was moderate.

Results observed for Ni are aligned with the results presented by Papazoglou et al. [43] that grew giant reed in soils irrigated with Ni-contaminated water in a two year experiment. However, results observed for Cd are different from the results also presented by Papazoglou et al. [43] in soils irrigated with Cd-contaminated water during the same two-year experiment. Using a solution containing the metals in varying concentrations (0, 5, 50, and 100 mg.L^{-1} of Ni and Cd in solution) to irrigate the pots, the total content of the metals in the soil was, at the end of the first year, 13.5 mg Cd kg^{-1} and 68.5 mg Ni kg^{-1}, and

973.8 mg Cd kg^{-1} and 2543.3 mg Ni kg^{-1} at the end of the second year. It was observed that the biomass yield suffered no significant changes for both heavy metals. However, the authors found that the heavy metals remained in the topsoil, reducing the plant's absorption. Atma et al. [41] also studied giant reed exposed to Ni contamination. For 35 days, the authors irrigated pots containing giant reed shoots with a solution containing 10, 50, and 100 mg Ni L^{-1}. It was notice that the high-level nickel irrigation affected the productivity of giant reed, as observed in the present study. Sabeen et al. [73] designed a pot experiment contaminating soils with a solution containing different levels of a Cd salt solution: 0, 50, 100, 250, 500, 750, and 1000 μg.L^{-1}. The study did not find significant differences in the plants' productivity but noticed a reduction in the tillers' number as the Cd level increased. Shaheen et al. [74] conducted a 30-day hydroponic experiment to test Cd's influence in giant reed growth, at 0, 25, 50, 75, and 100 mg Cd L^{-1}. The experiment confirmed the harmful impact of Cd in giant reed productivity, showing that the highest concentration of Cd contamination caused a decrease in biomass productivity of around 20%. However, it is difficult to make comparisons to these studies once there is no information on the amount of bioavailable Cd in the soil.

In terms of Cr contamination, Barbosa et al. [40] conducted a similar study with giant reed, testing 300 and 600 mg.kg^{-1} total Cr during two growing cycles; the bioavailable fractions were similar to the ones presented in the current study. However, Barbosa et al. [40] observed a reduction of only 30–40%, and in the present study, the biomass yield reduction was of 70%. As observed for switchgrass, the higher loss observed in this study, when compared with the study of Barbosa et al. [40], can be justified by the partial contamination of the soil with Cr (VI) [71].

According to Kabata-Pendias [67], there is no evidence as to the role of Cr and Ni in a plant's metabolism. Cr can affect the plant in different forms; it usually accumulates in the roots binding to cell walls. Its toxicity depends on its oxidation state, and in fact small concentrations of Cr in plants (1 or 2 mg.kg^{-1}) can be harmful towards the plants growth [67]. Chromium can affect the plant in different ways, reducing or even inhibiting the seed's germination and the growth of roots, stems, and leaves [75]. The photosynthetic apparatus can be damaged, as can the belowground organs, which causes retarded growth of the plants [67]. Nickel can be found in different oxidation states, however, the most toxic is Ni (II). Nickel is usually taken up by the roots and transported to the leaves and stems, where it can be stored [76]. Despite some hyper-tolerant and hyperaccumulator plants, like Berkheya coddi, that can reach Ni levels of 18,000 mg.kg^{-1} in its biomass, other Ni-sensitive plants, such as oats, can be affected by concentrations from 24–308 mg.kg^{-1} of metal in their biomass [67]. Nickel can lead to several hazardous effects to the plant (related with the plant's morphology, physiology, and biochemistry) [77]. In some cereals, excess Ni in the soil can be noticed by some plant characteristics, such as interveinal chlorosis affecting the new leaves, grey-green leaves affecting the aboveground biomass, and, additionally, brown and stunted roots in the belowground biomass [67]. Regarding Cd, although some enzymes depend on this metal to have regular activity, high concentrations of Cd in the soil causes root damage and growth retardation, interfering with protein synthesis, nutrient absorption, and photosynthesis. The absorption of Cd can be carried out passively by roots or metabolically. For sensitive plants, Cd levels of around 5 mg.kg^{-1} of Cd in biomass are already toxic, while concentrations around 20 mg.kg^{-1} can be critical for the crop's growth and development [67].

The belowground apparatus showed higher tolerance than the aerial fraction of the plants, either with switchgrass and giant reed. This behavior could be associated with the plants defense mechanisms, making it possible to accumulate the metals in the roots vacuoles [78], reducing the harmful effects of these metals, either in the belowground or in the aerial fraction. The development of tissue scarification and secondary sheath bundles, which can quench the metals, are mechanisms that can contribute to increase the tolerance of the plants [79]. Still, limited information is known regarding the interactions between the rhizomes and roots of these perennials and the growing medium, and more studies linked with them might provide hints on how these crops can become more tolerant to soils contaminated with heavy metals. The ratio of aboveground/belowground biomass is informative and shows a reduction due to contamination. The reduction in the aboveground biomass in the contaminated pots is responsible for this decrease, but in the Ni_{110} and Cr_{600} pots in the giant reed essay, and in the Cd_4 and Ni_{110} pots in the switchgrass essay, the reduction is also due to the increase in the belowground productivity. It could indicate a response of the crop to increase the plants' tolerance capacity [67] once the augmentation of the root apparatus leads to maintaining the aboveground biomass productivity. The ratio of leaves/stems in the second growth cycle of the giant reed essay did not suffer any significant changes due to the increase in Cd, Cr, and Ni contamination in the soils. This reflects the tolerance of this crop to the metals once the photosynthetic apparatus associated with leaves remained balanced with the stem's biomass.

The decrease in giant reed's productivity from the first to the second year regarding Cd and Cr contamination indicates that the accumulation and storage of the absorbed metals during first growth cycle could have damaged the belowground organs, reducing its capacity to regrowth normally in the second growing season. The situation was different in Ni trials. Since this heavy metal is usually translocated to aboveground biomass [76], the remaining Ni in the belowground organs did not affect the regrowth on the second growing cycle.

In this way, it is possible to observe that Cr trials were the most affected by the heavy metal, suppressing switchgrass growth and reducing giant reed productivity by 70%. For Ni and Cd trials, it was observed that when the heavy metal's concentration increases, the yield of both giant reed and switchgrass decreases. Regarding Cr contaminate trials, giant reed showed to be less sensitive to the contaminant's presence than switchgrass in terms of biomass production. For Cd and Ni in both contamination levels, the aerial biomass productivity was similar for both crops. On the other hand, the root system of giant reed presented a higher yield in all studied trials.

3.3. Biomass Composition

Table 4 presents the switchgrass's aerial part composition after the second growing cycle while Table 5 shows giant reed's biomass composition. Although the essay was conducted in pots, the differences among the biomass composition can indicate the effect of the contamination on the biomass quality for energy.

Table 4. Composition of switchgrass' aerial biomass.

	Control	**Cd_4**	**Cd_8**	**Ni_{110}**	**Ni_{220}**
Aerial Biomass					
Volatile Matter (% dw)	78.7 ± 0.5^{a}	75.9 ± 0.1^{b}	74.6 ± 0.1^{c}	73.0 ± 0.1^{d}	78.8 ± 1.2^{a}
Ash (% dw)	8.3 ± 0.3^{a}	7.5 ± 0.2^{b}	8.2 ± 0.2^{a}	8.1 ± 0.2^{a}	7.8 ± 0.8^{ab}
Fixed Carbon (% dw)	13.2 ± 0.4^{d}	16.8 ± 0.1^{c}	17.2 ± 0.2^{b}	18.8 ± 0.1^{a}	13.2 ± 2.4^{d}
HHV ($MJ.kg^{-1}$)	18.3 ± 0.3^{a}	18.7 ± 0.3^{a}	18.5 ± 0.2^{a}	18.6 ± 0.4^{a}	18.6 ± 0.8^{a}
N (%, dw)	0.71 ± 0.01^{d}	0.91 ± 0.08^{b}	1.11 ± 0.07^{a}	0.78 ± 0.03^{c}	0.89 ± 0.08^{bc}
P ($g.kg^{-1}$, dw)	0.52 ± 0.06^{b}	0.63 ± 0.14^{ab}	0.46 ± 0.12^{b}	0.63 ± 0.01^{a}	0.47 ± 0.08^{b}
K ($g.kg^{-1}$, dw)	11.6 ± 0.9^{a}	10.9 ± 0.9^{a}	10.8 ± 1.2^{a}	11.5 ± 0.7^{a}	11.3 ± 0.9^{a}
Ca ($g.kg^{-1}$, dw)	11 ± 2^{a}	12 ± 3^{a}	14 ± 3^{a}	12 ± 2^{a}	9 ± 2^{a}
Mg ($g.kg^{-1}$, dw)	10.6 ± 1.0^{a}	9.3 ± 0.9^{a}	8.8 ± 1.2^{a}	9.5 ± 0.7^{a}	8.4 ± 1.4^{a}
Na ($g.kg^{-1}$, dw)	1.09 ± 0.05^{ab}	0.99 ± 0.09^{b}	1.18 ± 0.08^{a}	0.99 ± 0.07^{b}	1.13 ± 0.08^{a}

dw: dry weight; Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter; For each parameter, different lower-case letters indicate statistical significance ($p < 0.05$) between treatments.

Table 5. Composition of giant reed aerial biomass.

	Control	**Cd_4**	**Cd_8**	**Cr_{300}**	**Cr_{600}**	**Ni_{110}**	**Ni_{220}**
Leaves							
Volatile Matter (% dw)	76.6 ± 0.7^{a}	74.9 ± 0.2^{b}	73.3 ± 0.4^{c}	74.2 ± 0.3^{b}	74.7 ± 0.3^{b}	76.6 ± 0.4^{a}	77.6 ± 1.1^{a}
Ash (% dw)	9.2 ± 0.4^{c}	10.4 ± 0.2^{b}	11.1 ± 0.2^{a}	11.0 ± 0.6^{ab}	11.6 ± 0.6^{a}	8.7 ± 0.4^{c}	9.4 ± 0.9^{bc}
Fixed Carbon (% dw)	14.5 ± 0.7^{ab}	14.8 ± 0.1^{b}	15.7 ± 0.6^{a}	15.3 ± 0.1^{a}	14.1 ± 0.1^{b}	15.1 ± 0.3^{a}	13.5 ± 1.1^{c}
HHV ($MJ.kg^{-1}$)	18.3 ± 1.1^{a}	18.0 ± 0.0^{a}	17.9 ± 0.1^{a}	18.0 ± 0.0^{a}	17.8 ± 0.1^{a}	18.4 ± 0.0^{a}	18.2 ± 0.1^{a}
N (%, dw)	1.7 ± 0.3^{a}	1.6 ± 0.2^{a}	1.9 ± 0.2^{a}	1.6 ± 0.2^{a}	1.6 ± 0.2^{a}	1.5 ± 0.2^{b}	1.7 ± 0.2^{a}
P (%, dw)	0.20 ± 0.01^{b}	0.46 ± 0.18^{a}	0.22 ± 0.04^{b}	0.26 ± 0.05^{b}	0.37 ± 0.06^{a}	0.23 ± 0.08^{b}	0.34 ± 0.05^{a}
K ($g.kg^{-1}$, dw)	7.4 ± 1.9^{b}	16.1 ± 2.6^{a}	18.2 ± 2.8^{a}	14.3 ± 2.4^{a}	10.9 ± 2.3^{b}	10.3 ± 2.4^{b}	8.2 ± 2.1^{b}
Ca ($g.kg^{-1}$, dw)	5.7 ± 2.1^{b}	5.4 ± 1.3^{b}	7.3 ± 2.0^{b}	9.7 ± 3.1^{ab}	6.4 ± 2.2^{b}	7.6 ± 2.1^{ab}	11.8 ± 2.1^{a}
Mg ($g.kg^{-1}$, dw)	4.0 ± 0.8^{a}	5.2 ± 1.2^{a}	6.5 ± 1.3^{a}	4.7 ± 1.3^{a}	6.5 ± 1.7^{a}	4.9 ± 1.5^{a}	7.5 ± 1.8^{a}
Na ($g.kg^{-1}$, dw)	2.0 ± 0.4^{a}	0.92 ± 0.27^{b}	0.85 ± 0.28^{b}	0.73 ± 0.23^{b}	0.86 ± 0.28^{b}	0.65 ± 0.13^{b}	0.99 ± 0.23^{b}
Stems							
Volatile Matter (% dw)	80.5 ± 0.0^{a}	75.9 ± 0.4^{b}	74.6 ± 0.2^{b}	77.1 ± 0.0^{b}	74.2 ± 0.2^{b}	81.4 ± 0.2^{a}	79.8 ± 0.2^{a}
Ash (% dw)	5.4 ± 1.3^{b}	6.2 ± 0.3^{b}	8.0 ± 1.4^{ab}	7.4 ± 0.4^{a}	6.2 ± 0.5^{b}	6.2 ± 0.6^{b}	7.4 ± 1.5^{ab}
Fixed Carbon (% dw)	15.1 ± 0.6^{c}	18.2 ± 0.3^{b}	18.3 ± 0.1^{b}	15.8 ± 0.3^{c}	19.4 ± 0.1^{a}	12.7 ± 0.2^{e}	13.7 ± 0.4^{d}
HHV ($MJ.kg^{-1}$)	19.1 ± 1.3^{a}	19.0 ± 0.1^{a}	18.8 ± 0.1^{a}	18.7 ± 0.2^{a}	19.0 ± 0.1^{a}	18.7 ± 0.2^{a}	18.7 ± 0.2^{a}
N (%, dw)	0.77 ± 0.05^{c}	1.6 ± 0.2^{a}	2.0 ± 0.3^{a}	1.0 ± 0.0^{b}	1.4 ± 0.3^{a}	1.3 ± 0.3^{b}	1.2 ± 0.5^{ab}
P ($g.kg^{-1}$, dw)	0.70 ± 0.10^{b}	1.9 ± 0.7^{a}	0.92 ± 0.25^{ab}	0.48 ± 0.21^{b}	0.87 ± 0.14^{b}	0.62 ± 0.30^{b}	0.58 ± 0.16^{b}
K ($g.kg^{-1}$, dw)	11.1 ± 2.6^{b}	15.8 ± 0.9^{b}	15.2 ± 0.8^{b}	16.4 ± 2.8^{ab}	19.6 ± 1.8^{a}	14.3 ± 2.3^{b}	15.8 ± 1.5^{b}
Ca ($g.kg^{-1}$, dw)	2.3 ± 0.1^{a}	2.6 ± 0.8^{ab}	2.0 ± 0.7^{ab}	2.4 ± 0.2^{a}	1.9 ± 0.2^{b}	2.6 ± 0.5^{ab}	2.4 ± 0.5^{ab}
Mg ($g.kg^{-1}$, dw)	3.0 ± 0.2^{a}	3.3 ± 0.7^{a}	4.4 ± 1.2^{a}	3.8 ± 1.3^{a}	4.3 ± 0.9^{a}	2.7 ± 0.8^{a}	3.2 ± 0.7^{a}
Na ($g.kg^{-1}$, dw)	1.8 ± 0.3^{a}	0.75 ± 0.16^{b}	0.87 ± 0.19^{b}	0.80 ± 0.23^{b}	0.77 ± 0.24^{b}	1.2 ± 0.4^{ab}	1.3 ± 0.3^{ab}

dw: dry weight; Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter; For each parameter, different lower-case letters indicate statistical significance ($p < 0.05$) between treatments.

Concerning switchgrass, in terms of HHV and ash content, contamination did not affect the values. In Cd_4 pots, the plants even presented a lower ash content compared with the control, and this reduction can be linked with the higher biomass production obtained, which may have induced a factor of dilution in the ash content. Despite the values of K, Ca, Mg, and Na remaining the same or lower, as observed in Cd_4 pots, the N and P content increased in the contaminated soils. In the case of P, this increment was noticed with significance in the Ni_{110} fields. In addition, the biomass exposure to Cd increased the nitrogen levels in the plant aerials' part by around 25% and 55% for Cd_4 and Cd_8, respectively, evidencing that the increase in Cd in the soil leads to an increase in N levels in the biomass. This relation was less sensitive for nickel, leading to a slight increase for the lower level, Ni_{110}, but to an increase of 20% in the higher level, Ni_{220}.

Regarding the results obtained with giant reed, it was observed that Cd and Cr contamination caused an increase in the ash content of giant reed leaves, but not Ni. In terms of stems, an increment on the ash content was observed in the Cr_{300} pots. In general, leaves showed to have a higher ash content compared to stems, and a higher amount of ash content contributed to a reduction in the HHV of the leaves compared with the stems. However, the contaminants did not affect the HHV in the leaves nor the stems. In terms of nitrogen, leaf content was not affected by the contamination, but the content in stems increased significantly due to the contamination. Phosphorus content also increased in the leaves due to Cd_4, Cr_{600}, and Ni_{220} contamination, and in the stems due to Cd_4 contamination. The N and P contents in leaves were also, in average, higher than the stems' concentration. In terms of the K and Ca content, a trend to a higher concentration in the contaminated pots was noticed, either in the leaves and/or in the stems. Magnesium content was not affected by the contamination, and the sodium content decreased due to the contamination in both leaves and stems. Leaves also presented higher Mg and Na than stems, but the K and Na content did not show a clear pattern between both fractions.

Biomass composition, e.g., ash and HHV, is essential in determining its potential utilization in thermochemical processes. The ash content and the HHV of switchgrass measured by Hu et al. [80] showed that the conversion of switchgrass in biofuels and bioenergy could be an option. In general, neither giant reed nor switchgrass suffered any alterations in HHV when compared with the literature (in the range 16.4–21.9 $MJ.kg^{-1}$ for switchgrass [81,82] and in the range 17.2 and 19.0 $MJ.kg^{-1}$ for giant reed [83,84]. Understanding that switchgrass ash content and giant reed stems ash content were not affected by contamination (except in the Cr_{300} pots of giant reed) is a promising result, and shows that for the tested contaminations, there will be no further load in ash residue when using contaminated biomass in thermochemical purposes. However, the increment of ash in the leaves due to contamination can make the use of those fractions not feasible. Ash values obtained for switchgrass and giant reed are also aligned with the ones presented in literature (for giant reed stems, in the range 4.8–7.4% [33,85], and 3.9–8.2% for switchgrass [86,87]), which is interesting, considering that the biomass obtained in this study is harvested from pots (so, presenting lower growth) and those expressed in literature are from field studies.

The increase in nitrogen levels witnessed in switchgrass and giant reed stems due to the heavy metals may represent an obstacle for the utilization of contaminated biomass in thermochemical processes. The gases generated in these processes are directly related to human and environmental problems [88], and an increment in the emissions due to contamination may represent a limitation for their use in pyrolysis, gasification, and combustion plants [89,90]. However, despite the increase in switchgrass and giant reed stems' N content, their biomass can be used in pyrolysis and combustion processes since the maximum N content in these processes should be 2.5% (pyrolysis) and up to 3% (domestic stoves or pellet burners for heat) or up to 15% (fixed bed combustion) [89,90]. For gasification, however, some processes–e.g., bubbling fluidized beds, dual fluidized beds–limit the N content of the feedstock to 1% [89,90], which will limit the use of giant reed stems harvested from contaminated soils (with the exception of giant reed from Cr_{300} pots) and the switchgrass collected from Cd_8 pots. Processes such as circulating fluidized beds for CHP (combined heat and power, gas engine) and circulating fluidized beds for syngas production have a higher limit of 2% in N content, and those processes can be applied to the biomass harvested from contaminated soils that exceed the limit of 1% N. Giant reed N content observed in this experiment is in agreement with literature that reports values in the range from 0.3 to 1.5% [83,91,92]. Only biomass stems from Cd pots showed a higher value than the range presented. This relation between Cd and N may be a consequence of the strong synergic interaction between these two elements, which is due to the formation of very stable complexes between proteins and Cd. In fact, Cd has high electronegativity values and can bond easily with sulfur present in proteins [67]. Therefore, the presence of Cd in the soil, can stimulate the uptake and mobilization of N in the plant. For switchgrass, the values observed in this experiment are also in line with what is observed in literature, with values ranging from 0.35 to 0.88% [86,87]. Interestingly, the same relation between Cd and N was also observed with switchgrass in Cd-contaminated pots, where switchgrass biomass presented a higher N content than the values presented in literature and in control pots.

The contents in terms of P, K, Ca, Mg, Na, and other elements are also important to evaluate in the potential of the biomass to be used in thermochemical processes. Indeed, they affect fly ash emissions, deposit formation, and ash handling/utilization/disposal. The values for these elements varied among the different biomasses. For switchgrass, according to the work of Monti et al. [93], the amount of those elements in terms of $g.kg^{-1}$ are in the following ranges, 0.25–0.77 (P), 1.50–3.56 (K), 1.10–8.20 (Ca), 1.02–2.71 (Mg), and 0.32–0.87 (Na). Compared to these results, the biomass that we collected from the pot essays presented, for K, Ca, Mg, and Na, higher values, and for P, similar values. For giant reed, the same work of Monti et al. [93] reported the following contents of those elements in stems: 0.32 (P), 5.61 (K), 0.97 (Ca), 1.03 (Mg) and 0.13 (Na), in $g.kg^{-1}$. As mentioned for switchgrass, the contents reported in the stems of giant reed harvested from the current pot essay showed consistently higher values than those reported by Monti et al. in their study [93]. The higher concentration of elements reported in the current work compared to the literature may be derived from the fact that the biomass was harvested from pots and not from field. Therefore, the biomass production is lower, and those macro-elements may be more concentrated. The differences observed may have also resulted from the soil type, the stage of growth, type of plant tissue, environment, cultural practices, etc. [86,93].

Considering the elements K and Na, a high amount in the biomass is linked to the damage of the pieces of equipment such as pipes and furnaces due to corrosion processes. Potassium and sodium also remain in the ash, decreasing its melt point and provoking its volatilization [94]. This can damage the combustion chambers (through sintering or slag formation), provoking a decrease in its lifetime and compromising the availability of the plant [95]. The results obtained in the study provide an indication that contamination did not affect the K content of switchgrass and giant reed stems (except for stems of giant reed obtained in Cr_{600} pots). Regarding giant reed leaves, the trend of an increase in K content with contamination that was significant in Cd pots (all levels of contamination) and Cr_{300} pots was observed. In terms of Na content, the contamination reduced the amount in giant reed stems and leaves; in switchgrass, the contents were similar to control. Table 6 presents the alkali index calculated for both crops in control and contaminated pots.

Table 6. Alkali index (Na_2O + K_2O, kg/GJ) of switchgrass and giant reed stems in control and contaminated pots.

	Control	**Cd_4**	**Cd_8**	**Cr_{300}**	**Cr_{600}**	**Ni_{110}**	**Ni_{220}**
Giant reed stems	0.8 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.3 ± 0.1	1.0 ± 0.2	1.1 ± 0.1
Switchgrass	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	-	-	0.8 ± 0.1	0.8 ± 0.1

Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

To determine the propensity of a fuel to slagging and fouling, Tortosa Masiá and collaborators [63] presented an alkali index (AI) that relates the amount of Na and K in the biomass per unit of energy with the probability of slagging and fouling formation through the thermochemical conversion of biomass. According to the authors, the AI can be classified in terms of indication for slagging and fouling: 0.17 < AI < 0.34—probable; AI > 0.34, slagging and fouling is virtually certain to occur. According to these, the values of the alkali index presented in Table 6 indicate that biomass from pots, either giant reed and switchgrass, have a high probability to cause slagging and fouling once results are higher than 0.34–even the biomass from control pots. These results are in line with what is considered a problem when perennial grasses are exploited through thermochemical processes, especially due to the high potassium content present in those biomasses, which is significantly higher than what is observed in woody biomass. Vassilev et al. [96] indicate a mean of 10.75 of K_2O (% weight to total ash) in wood and woody biomass and a mean of 26.65 of K_2O (% weight to total ash) in herbaceous and agricultural biomass. Both the occurrence of slagging and fouling are a challenge to thermochemical conversion of biomass since the ash particles melt and accumulate in the walls of furnaces and pipes, reducing the temperature inside the furnace and the oxidation of the fuel, increasing the emission of CO [97,98]. Both crops showed a lower fuel quality when compared to coal or pine chips that have AI values of 0.04 and 0.17, respectively [63]. However, they have a similar AI index to olive residues (1.14) [63]. This indicates that in order to proceed with the thermochemical valorization of these biomasses, some treatment must be applied, specially to reduce the fouling formation trend (linked with K and Na contents). Comparing the data obtained for control and contaminated pots, it is interesting not to report differences in AI for switchgrass. In the case of giant reed stems, AI increased due to contamination, but this increment was more problematic in Cr_{600} pots.

The fiber content of the samples can be observed in Table 7. The analysis of giant reed was only made for the stems and not for the leaves, considering that the stems will be the fraction to be valorized.

Table 7. Composition of giant reed stems and switchgrass aerial biomass in hemicellulose, cellulose, and lignin (% *w*/*w*, dry weight).

		Hemicellulose	Cellulose	Lignin	Total Fiber
Giant reed stems	Control	31.5 ± 1.4	25.6 ± 5.1	20.0 ± 1.7	77.1 ± 2.0
	Cd_4	29.7 ± 1.0	27.8 ± 4.5	21.2 ± 3.1	78.7 ± 2.5
	Cd_8	30.3 ± 4.7	26.5 ± 1.7	20.8 ± 2.7	76.7 ± 1.7
	Cr_{300}	30.4 ± 1.3	19.5 ± 2.3	22.5 ± 1.4	72.3 ± 0.5
	Cr_{600}	30.7 ± 1.1	22.5 ± 1.3	21.9 ± 1.4	75.1 ± 1.1
	Ni_{110}	30.1 ± 0.7	26.3 ± 0.7	19.5 ± 1.9	75.9 ± 0.5
	Ni_{220}	30.3 ± 2.0	25.1 ± 3.0	19.1 ± 0.4	74.5 ± 1.3
Switchgrass	Control	34.6 ± 0.7	22.3 ± 0.1	15.2 ± 1.4	72.1 ± 2.0
	Cd_4	35.6 ± 6.9	23.5 ± 1.6	14.9 ± 0.5	74.0 ± 5.7
	Cd_8	34.0 ± 1.8	25.2 ± 1.0	14.7 ± 0.5	73.8 ± 1.3
	Ni_{110}	30.0 ± 2.1	22.9 ± 4.8	18.1 ± 3.5	71.0 ± 0.8
	Ni_{220}	33.4 ± 1.4	23.2 ± 5.5	19.8 ± 7.0	76.3 ± 0.1

Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

The results of fiber content indicate that for both giant reed and switchgrass, the hemicellulose content is the highest fraction, followed by cellulose and lignin. This trend in the fiber composition of biomass was extensively reported in literature for both giant reed and switchgrass. For giant reed, the literature reports cellulose content from 29–44%, hemicellulose from 13–36%, and lignin from 11–34% [83,84,99–101]. For switchgrass, the literature reports cellulose content from 32–40%, hemicellulose from 19–32%, and lignin from 7–23% [70–82,102,103]. The results from the current study are aligned with the data presented, but the cellulose content obtained in both crops was somehow lower than the values reported, and hemicellulose content reported in switchgrass was similar or higher than the values reported by literature. This difference can be attributed to the fact that the biomass of the current study was obtained in pots, and data from literature is of biomass harvested from fields. In pots, the growth of the biomass is limited by the size of the pots, and the processes of lignification and cellulose formation are delayed, which causes a higher production of hemicelluloses and lesser of cellulose. In giant reed, the cellulose and lignin content are higher than in switchgrass, however the hemicellulose content is lower. What is interesting to report is the fact that contaminated soils did not disturb the fiber content of switchgrass and giant reed stems ($p < 0.05$), which represents an opportunity for the use of this biomass. The high value of fiber content indicates prospects for the valorization of those biomasses in biorefinery processes. All three fractions presented appealing values for their separation and transformation into bioproducts.

3.4. Heavy Metal Concentration in Switchgrass and Giant Reed

Table 8 presents the heavy metals content for the studied crops (giant reed and switchgrass) after the second growing cycle. Results indicate that, except for Ni in the giant reed leaves, the increment in heavy metal concentration increases the amount of element in each fraction of each crop. In terms of the distribution between aboveground biomass and belowground biomass, a higher Cd and Ni concentration was observed in the belowground fraction of switchgrass, but in terms of Cr (in control plants), a higher content was observed in the aboveground fraction.

Table 8. Heavy metals concentration (mg.kg^{-1}, dw) in the different biomass fractions of giant reed and switchgrass.

Species	Treatment	Element Analyzed			
Switchgrass			**Aboveground Fraction**	**Belowground Fraction**	
	Control	Cadmium	0.33 ± 0.01 [b]	1.98 ± 0.02 [c]	
	Cd_4		1.09 ± 0.14 [a]	5.0 ± 0.6 [b]	
	Cd_8		1.33 ± 0.01 [a]	10.6 ± 1.2 [a]	
	Control	Chromium	16.2 ± 1.9	8.8 ± 3.0	
	Cr_{300}		-	-	
	Cr_{600}		-	-	
	Control	Nickel	8.0 ± 2.0 [b]	21 ± 3 [b]	
	Ni_{110}		12.4 ± 3.8 [ab]	53 ± 3 [a]	
	Ni_{220}		14.7 ± 1.1 [a]	58 ± 2 [a]	
Giant reed			**Leaves**	**Stems**	**Rhizomes**
	Control	Cadmium	0.97 ± 0.30 [b]	0.28 ± 0.18 [c]	0.61 ± 0.04 [c]
	Cd_4		3.1 ± 0.7 [a]	0.91 ± 0.21 [b]	1.88 ± 0.09 [b]
	Cd_8		4.3 ± 0.7 [a]	4.4 ± 0.6 [a]	4.6 ± 1.5 [a]
	Control	Chromium	20 ± 6 [b]	7.5 ± 2.1 [b]	29 ± 3 [c]
	Cr_{300}		28 ± 7 [b]	9.1 ± 1.0 [b]	245 ± 70 [b]
	Cr_{600}		92 ± 11 [a]	15.6 ± 0.8 [a]	744 ± 94 [a]
	Control	Nickel	96 ± 14 [a]	5.45 ± 0.02 [c]	41 ± 7 [c]
	Ni_{110}		77 ± 10 [a]	9.6 ± 0.1 [b]	54 ± 6 [b]
	Ni_{220}		90 ± 16 [a]	15.1 ± 0.1 [a]	134 ± 10 [a]

dw: dry weight; Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter; For each species, biomass fraction and element analysed, different lower-case letters indicate statistical significance ($p < 0.05$) between treatments.

In the case of giant read, a trend was observed in which the roots/rhizomes and leaves presented a higher heavy metal concentration (although in Cr-contaminated pots, the content of Cr in leaves is significantly lower than in rhizomes). Stems are the fraction of the plant that presented the lowest content of heavy metals (in Cd, differences to leaves and rhizomes are not significant). Between species, Cd concentration was higher in the aboveground fractions of giant reed and in the belowground fraction of switchgrass. In terms of Cr, the comparison can only be made with control plants, and results indicate that similar values can be found for giant reed and switchgrass. In terms of Ni, giant reed leaves present a higher concentration than switchgrass aboveground material, but the concentration of Ni in the stems of giant reed is similar to the content observed for aboveground switchgrass. The Ni concentration in the belowground of both crops is similar to the control and Ni_{110} level of contamination. However, at higher levels of contamination, giant reed presents a higher content of Ni.

The results presented followed the same pattern of other data presented in the literature. The levels of Cd in switchgrass biomass were studied by Reed et al. [104] for different concentrations and pH, despite the studied concentrations being much higher than the ones used in this work (to a maximum of 200 mg Cd.kg^{-1}). An increase in Cd accumulation was observed led by the increase in the content in Cd in the soil when decreasing soil pH. The increment in Cd in the above- and belowground biomass of switchgrass, with the increment of Cd in the soil, was also reported in the current study. In the study of Reed et al., the accumulated Cd in roots (to a maximum of 900 mg.kg^{-1}) was also considerably higher than in the aerial part of the biomass for all treatments (reaching a maximum of 270 mg.kg^{-1}) [104]. The same pattern was observed in the current study, where the belowground biomass reached 10.6 mg.kg^{-1} and the aerial biomass reached only 1.33 mg.kg^{-1}. The study of Reed et al. showed much higher Cd content in the aerial and belowground biomasses due to the higher Cd content tested in the soil and also due to the extremely low pH of the soil (4.1)–very acidic, and thus promoting the mobilization of Cd in the soil [104].

The concentration of Cd in giant reed was also a study theme for Sabeen et al. [73]. The authors observed that Cd content increased in all fractions of the plant with the increment of Cd in soil, as it was observed in the current study. In the current study, no significant differences can be observed among fractions of the plant in terms of Cd concentration, particularly in the higher level of contamination; the same was observed in the work of Sabeen et al. [73]. The concentration of Cd reached a maximum of 220 $mg.kg^{-1}$ in the belowground biomass, 190 $mg.kg^{-1}$ in the stems, and 140 $mg.kg^{-1}$ in the leaves [73]. Again, the work of Sabeen et al. [73] presented a much higher content in Cd in all fractions of giant reed than what was reported in the current study, but this is because the concentration of Cd in the soil medium was also much higher (to a maximum of 300 $mg.kg^{-1}$), when in the current work the maximum concentration tested was 8 $mg.kg^{-1}$. Other differences between the current study and the studies of Reed et al. [104] and Sabeen et al. [73] are that those studies were carried out over a shorter period, 45–60 days and 21 days, respectively, and the current study analyzed the biomass after the second harvest.

Ni accumulation in giant reed was also studied by Atma et al. [41], who observed that the increase in the concentration of this heavy metal in the soil increased the heavy metal in the plant, especially in the roots [41]–similar to the results obtained in the presented study. Indeed, the concentration of Ni in giant reed roots when exposed to a watered solution containing 100 $mg.L^{-1}$ of Ni reached a maximum of 80 $mg.kg^{-1}$, although the difference to the content when the crop was watered with a 10 mg $Ni.L^{-1}$ solution was not significant. In the current study, rhizomes of giant reed reached a maximum of 134 $mg.kg^{-1}$ when the soil was contaminated with 220 $mg.kg^{-1}$. In the study by Atma et al. [41] on giant reed watered with Ni solutions and fertilized with NPK, it was reported that Ni content in the leaves (34–80 $mg.kg^{-1}$) and in the stems (20–60 $mg.kg^{-1}$) did not show a significant difference between 10 and 100 $mg.L^{-1}$ of Ni. In the current study, the Ni content in the leaves did not change significantly with contamination (77–96 $mg.kg^{-1}$), and the value was similar to the study of Atma et al. However, in the case of stems, it showed an incremental increase with the Ni increment in soil (to a maximum of 15 $mg.kg^{-1}$, when giant reed was exposed to 220 $mg.kg^{-1}$), and the content was much lower than what was observed in the Atma et al. study; again, the Atma et al. study was performed in a much shorter period of time–only 36 days. To our knowledge, no studies were performed with Ni and switchgrass, and therefore the current study is pioneering the demonstration of how this crop behaves when facing this type of contamination.

Concerning the study of the effect of Cr contamination in the switchgrass and giant reed contents in Cr, the comparison to other studies can only be made with data obtained for giant reed, since no production of switchgrass was obtained in the Cr-contaminated pots.

In a similar study using the same Cr concentrations (Cr_{300} and Cr_{600}), giant reed Cr content increased in all structures, especially in the belowground ones, with the increment in Cr in the soil [40]. This pattern was also observed in the current study. However, the maximum content observed in the present work in the belowground fraction (744 $mg.kg^{-1}$) and in leaves (92 $mg.kg^{-1}$) and stems (16 $mg.kg^{-1}$) is by far highest than in the study by Barbosa and collaborators (only a maximum of 34 $mg.kg^{-1}$ for rhizomes and 13 $mg.kg^{-1}$ for the above ground fraction) [40]. The difference can be attributed to the salts of Cr used to artificially contaminate the soil–namely the application of Cr (VI), which is more mobile in the soil and therefore more prone to be absorbed by the belowground organs of the plant.

Cadmium, chromium, and nickel are usually minor components of the ash. However, when using biomass harvested from contaminated soils, the values present in the biomass can significantly increase and represent a major component of the ash. Moreover, cadmium is an easily volatile element and can be disengaged from the biomass into the released gases reacting there in thermochemical processes [95]. These issues may cause further obstacles for its use, and knowledge of how it interferes with thermochemical processes must be safeguarded.

3.5. Phytoremediation Indexes

The phytoremediation indexes of giant reed and switchgrass are presented in Tables 9 and 10, respectively.

Table 9. Modified accumulation index (mAI), modified bioconcentration factor (mBCF), modified bioaccumulation factor (mBAF), translocation factor (TF), and modified translocation factor (mTF) of giant reed under Cr-, Cd-, and Ni-contaminated soils.

Structure	Trial	mAI	mBCF	mBAF (%)	TF (Aboveground/ Belowground)	mTF (Aboveground/ Belowground)
Stems	Cr_{300}	0.38 ± 0.20	0.14 ± 0.02	0.003 ± 0.001	0.15	0.01
	Cr_{600}	0.69 ± 0.35	0.11 ± 0.01	0.002 ± 0.001	0.38	0.03
	Ni_{110}	1.32 ± 0.27	0.11 ± 0.01	0.005 ± 0.001	0.77	0.12
	Ni_{220}	1.38 ± 0.55	0.08 ± 0.00	0.003 ± 0.001	0.93	0.13
	Cd_4	2.31 ± 1.73	0.22 ± 0.06	0.010 ± 0.003	0.32	0.05
	Cd_8	5.19 ± 3.79	0.44 ± 0.06	0.009 ± 0.003	0.46	0.06
Leaves	Cr_{300}	0.39 ± 0.18	0.42 ± 0.10	0.021 ± 0.007		
	Cr_{600}	1.59 ± 0.79	0.63 ± 0.09	0.039 ± 0.015		
	Ni_{110}	0.60 ± 0.21	0.86 ± 0.13	0.115 ± 0.032		
	Ni_{220}	0.42 ± 0.13	0.49 ± 0.09	0.040 ± 0.009		
	Cd_4	2.19 ± 0.97	0.73 ± 0.18	0.091 ± 0.027		
	Cd_8	1.58 ± 0.73	0.43 ± 0.07	0.028 ± 0.009		
Roots	Cr_{300}	1.09 ± 0.40	2.30 ± 0.25	1.869 ± 0.547		
	Cr_{600}	1.89 ± 0.70	1.30 ± 0.17	1.493 ± 0.463		
	Ni_{110}	1.16 ± 0.45	0.86 ± 0.18	1.015 ± 0.293		
	Ni_{220}	0.76 ± 0.35	0.41 ± 0.09	0.326 ± 0.123		
	Cd_4	1.36 ± 0.44	1.85 ± 0.35	1.930 ± 0.462		
	Cd_8	1.04 ± 0.37	0.95 ± 0.14	0.627 ± 0.168		

Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

Table 10. Modified accumulation index (mAI), modified bioconcentration factor (mBCF), modified bioaccumulation factor (mBAF), translocation factor (TF), and modified translocation factor (mTF) of switchgrass under Ni- and Cd-contaminated soils.

Structure	Trial	mAI	mBCF	mBAF (%)	TF	mTF
Aboveground	Ni_{110}	1.77 ± 0.96	0.14 ± 0.04	0.032 ± 0.011	0.23 ± 0.07	0.15 ± 0.07
	Ni_{220}	0.68 ± 0.35	0.08 ± 0.01	0.006 ± 0.002	0.25 ± 0.02	0.17 ± 0.06
	Cd_4	3.67 ± 1.85	0.26 ± 0.05	0.059 ± 0.014	0.22 ± 0.04	0.16 ± 0.06
	Cd_8	2.14 ± 1.42	0.13 ± 0.01	0.015 ± 0.007	0.13 ± 0.02	0.09 ± 0.05
Belowground	Ni_{110}	4.73 ± 1.84	0.59 ± 0.05	0.210 ± 0.067		
	Ni_{220}	1.69 ± 0.54	0.32 ± 0.01	0.037 ± 0.008		
	Cd_4	4.20 ± 1.61	1.19 ± 0.20	0.376 ± 0.134		
	Cd_8	4.07 ± 0.93	1.07 ± 0.14	0.155 ± 0.023		

Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

Plants' phytoremediation potential are associated with their capability to remediate contaminated soils. This process occurs through the absorption and accumulation of the contaminants in their organs [40], stimulating the remediation of pollutants through the release of enzymes and exudates, or stimulating microorganisms in the soil–roots interface [40,105]. When analyzing phytoextraction, several points must be taken into consideration: (a) the influence of the contaminant in the plant's growth that TI indicates, (b) the capability of the plant to absorb and store the pollutants when exposed to a higher than usual amount, shown by the mAI, (c) the capability of the plant to accumulate the contaminants, presented by mBCF and mBAF, and (d) the potential of the plant to transfer the accumulated contaminants from the belowground to the aboveground fraction of the crop, indicated by the TF and mTF.

The modified accumulation index (mAI) shows the plant's potential to extract and accumulate heavy metals when exposed to higher concentrations than usual. Thus, mAI indicates that giant reed's ability to accumulate Cd is higher than for the other studied heavy metals. The same was observed for switchgrass, and switchgrass on average also presented a higher mAI than giant reed, a fact that is interesting when planning a phytoremediation action.

The indication of which part of the plant is used to store the heavy metals is described by the mBCF. It can be observed that giant reed accumulates Cr and Cd mainly in the belowground part, while Ni is accumulated both in the belowground structure and leaves, in similar ratios. Switchgrass accumulation of Ni and Cd also occurs primarily in the belowground biomass. On average, giant reed presented a higher mBCF for Ni and Cd than switchgrass, probably due to lower productivity and higher metal content.

The capability of the plant to phytoremediate the soil is indicated by mBAF. Results suggest that, for both giant reed and switchgrass, the remediation is mainly promoted by the belowground biomass, indicating a trend for these crops to promote the phytostabilization of heavy metals. In terms of the belowground fraction, indexes were higher for giant reed than for switchgrass. Concerning the aboveground fractions, the leaves of giant reed also show higher indexes than stems of giant reed and aboveground fractions of switchgrass, for Ni and Cd.

The values of TF and mTF translate the crops' potential to relocate the contaminants to the aboveground biomass. A TF value higher than 1 indicate that the contamination is stored mainly in the aboveground part of the plant, while the mTF combines the TF with the production of biomass. The highest accumulation of the contaminants in the aerial part is interesting from the phytoextraction point of view. Once the contaminants are in the aerial part, the biomass harvest also removes the soil pollutants. Giant reed TF results indicate that this crop had difficulty in moving Cr and Cd to the aerial parts, making this plant more suitable for Cr and Cd phytostabilization. However, Ni has moved to the aerial parts with great ease. Therefore, the plant can translocate a significant amount of the contamination to the aerial part, enabling this contaminant to be harvested together with the crop. However, when combined with the biomass yield, translated by mTF, Ni presented less advantages to phytoextraction. Switchgrass was shown to not be a very suitable crop for phytoextraction processes regarding Ni and Cd contamination, having a very low TF and mTF, which indicate that the plant transfers these contaminants to the aboveground part at a low rate. Having a low mTF can be interesting, not from the phytoextraction potential, but from the potential of using the aerial biomass for energy purposes.

3.6. Heavy Metal Content in Percolated Waters

Another important value is the amount of heavy metals in the ground that can be leached, contaminating belowground water. Results obtained in this study are presented in Table 11. According to the results presented, the amount of heavy metals percolated increased with the artificial contamination of the soils. However, all the values obtained do not exceed the limit values in the discharge of wastewaters (0.2 mg.L^{-1} Cd, 2.0 mg.L^{-1} Cr and 2.0 mg.L^{-1} Ni), except in the trials with giant reed with the highest level of contamination of Cr [106]. A comparison made with the heavy metal content in percolated waters obtained from pots without plants (results not presented) indicate that the soil–biomass system trapped a higher quantity of metals than the soil system itself, in the case of Cd and Ni contamination. This indicates that those perennial grasses can retain and stabilize the soil contaminants, reducing its percolation to groundwaters. A similar result was obtained by Costa et al. [107], where the plant–soil system withheld more than 90% of the contaminants from wastewaters. Concerning the Cr-contaminated pots, interestingly, the pots of switchgrass that did not have any biomass, liberated in Cr_{600} pots, had less Cr than the giant reed pots with the same level of Cr contamination, and this amount was similar to what was obtained in pots without plants. In the pots with giant reed, with 600 mg.kg^{-1} Cr, the amount percolated was higher than what was observed in switchgrass pots and with pots without plants. This increased amount of Cr in the percolates, in the presence of giant reed, can be related to the higher oxidation of Cr (III) to Cr (VI), which presents higher solubility in pore water and soil. Hexavalent Cr is also not easily absorbed by soil colloids, thus increasing its content in the percolated waters. The increased oxidation process, in the presence of giant reed, can be conducted by the manganese (Mn) oxides and the dissolved oxygen (O_2) [71]. Rhizomes and roots of giant reed can release root exudates such as organic acids, complexing with Cr (III) and enhancing its solubility and mobility in soil [108]. However, both explanations for the result obtained in the giant reed pots most contaminated with Cr need confirmation with more experiments. Significant differences were noticed when comparing the two species, also in the Cd- and Ni-contaminated pots. Indeed, before the second harvest, switchgrass pots presented a lower amount of percolated Cd and Ni content than giant reed pots, especially in the higher contaminated level. This difference may be also a consequence of the release of exudates from the belowground fraction of giant reed that enhances its solubility in the soil column; but again, more experiments are needed to help clarify the current data.

Table 11. Heavy metals content in percolated waters.

Main Element of Contamination	Crop	Heavy Metal in Percolated Waters ($mg.L^{-1}$)			Limit Values in the Discharge of Wastewater [96]
		Control	Low	High	
Cd	Switchgrass	0.048 ± 0.007^{aB}	0.059 ± 0.012^{bAB}	0.076 ± 0.012^{bA}	0.2 $mg.L^{-1}$ Cd
	Giant reed	0.031 ± 0.008^{aB}	0.111 ± 0.012^{aA}	0.105 ± 0.015^{aA}	
Cr	Switchgrass	0.418 ± 0.015^{aB}	1.008 ± 0.101^{aA}	0.632 ± 0.156^{bB}	2.0 $mg.L^{-1}$ Cr
	Giant reed	0.399 ± 0.015^{aC}	1.034 ± 0.056^{aB}	4.344 ± 0.225^{aA}	
Ni	Switchgrass	0.036 ± 0.007^{aB}	0.128 ± 0.032^{aA}	0.082 ± 0.016^{bA}	2.0 $mg.L^{-1}$ Ni
	Giant reed	0.048 ± 0.008^{aC}	0.105 ± 0.018^{aB}	0.488 ± 0.062^{aA}	

Low and high correspond to the lower and the higher tested artificial contamination; for each metal and crop, different upper-case letters indicate statistical significance ($p < 0.05$) between treatments; for each metal and treatment, different lower-case letters indicate statistical significance ($p < 0.05$) between crops.

3.7. Global Evaluation of Switchgrass and Giant Reed Energy Potential when Cultivated in Cd-, Cr-, and Ni-Contaminated Soils

To understand the thermochemical potential of each crop when cultivated in Cd-, Cr-, and Ni-contaminated soils, a global evaluation was assessed. For this, a relation between yield and HHV was made, and also between yield and two characteristics of the biomass–ash and N content–which are important and determinant in terms of fuel quality. In addition, to evaluate the phytoremediation potential, a relation was also made between the yield and the %mBAF (Figures 4 and 5). In this evaluation, only the stems of giant reed and the aboveground fraction of switchgrass were considered as feedstock for energy.

Evaluation of the energy potential of switchgrass when cultivated in Cd- and Ni-contaminated soils (Cr contamination in the soils inhibited the biomass production) shows that the highest biomass feedstock can be obtained in the control soils (non-contaminated) and in the soils with lower contamination levels (Ni_{110} and Cd_4) (Figure 4). Lower yields were obtained in soils with higher contamination. Lower yields will reduce the energy, costs, and greenhouse savings, as was demonstrated in the work of Gomes and collaborators [109]. Pots presenting lower yields also presented lower phytoextraction potential, measured through the mBAF index (so, in Cd_8 and Ni_{220} soils, the phytoextraction capacity of switchgrass was lower). Switchgrass presented a higher mBAF to Cd, and lower to Ni. Contamination did not affect the biomass composition in terms of HHV and ash content, but nitrogen content increased with contamination, especially in the case of the biomass harvested in Cd_8 soils that presented a higher value.

Evaluation of the giant reed stems energy potential when cultivated in Cd-, Cr-, and Ni-contaminated soils shows that the highest biomass feedstock can be obtained in the control soils (non-contaminated), followed by soils with lower contamination levels of Ni and Cd (Ni_{110} and Cd_4, respectively) (Figure 5). Lower yields were obtained in soils with higher contamination levels of Ni and Cd, and in Cr-contaminated soils (high and low contamination level). Giant reed presented a higher mBAF to Cd, followed by Ni, as was also observed for switchgrass. Plants harvested from Cr-contaminated pots presented the lowest mBAF of the three heavy metals tested. In the Cd and Ni pots, the increase in the contamination reduced the mBAF, mainly caused by the reduction in yields. In the Cr pots, no differences were obtained for the mBAF between both doses of contamination. Contamination did not disturb the biomass HHV, but the biomass ash and nitrogen contents were affected by the contamination. A lower ash content was observed for the plants from the control. A higher ash content was observed with higher levels of contamination of Cd and Ni. Chromium contamination also induced a higher ash content, but differences between both levels of Cr contamination were not identified. Figure 5A shows a correlation between yields and ash content when considering Ni and Cd contamination.

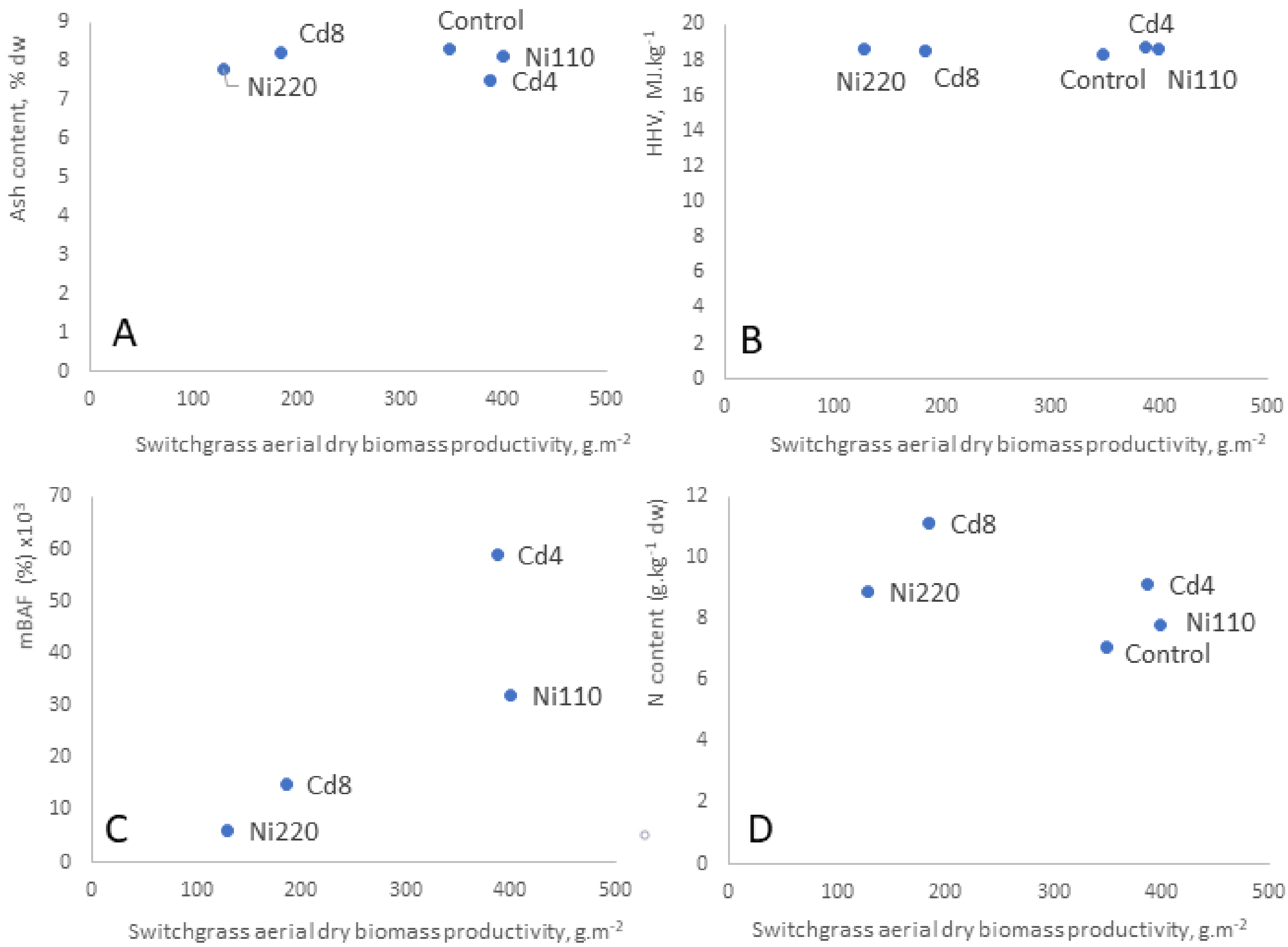

Figure 4. Global evaluation of switchgrass energy potential when cultivated in Cd- and Ni-contaminated soils. Yields (g/m^2) are related with ash content (% dry weight, dw) (**A**), HHV (MJ/kg) (**B**), % mBAF (modified bioaccumulation factor) (**C**), and nitrogen content (g.kg^{-1} dry weight, dw) (**D**). Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

A lower nitrogen content was also observed for the plants from the control. An increase in N content was observed with the increase in contamination of Cd and Cr. Nickel contamination also induced a higher N content, but differences between both levels of Ni contamination were not identified. Figure 5D shows a correlation between yields and N content when considering Cd contamination. In pots contaminated with Ni or Cr, this correlation was not identified.

In terms of phytoremediation, the extraction of the studied metals from the soil by both crops is very low: for switchgrass, <1% and for giant reed, <2%. However, in terms of phytoremediation, the effect of the presence of vegetation in contaminated soil is beneficial from several other points. The respiration of the soil increases, and the organic matter of the soil and structure of the soil improves. The microfauna also increases, and this can be beneficial for improving the tolerance of the crop to the contamination. Those facts were observed in our study (data not shown), both with giant reed and with switchgrass.

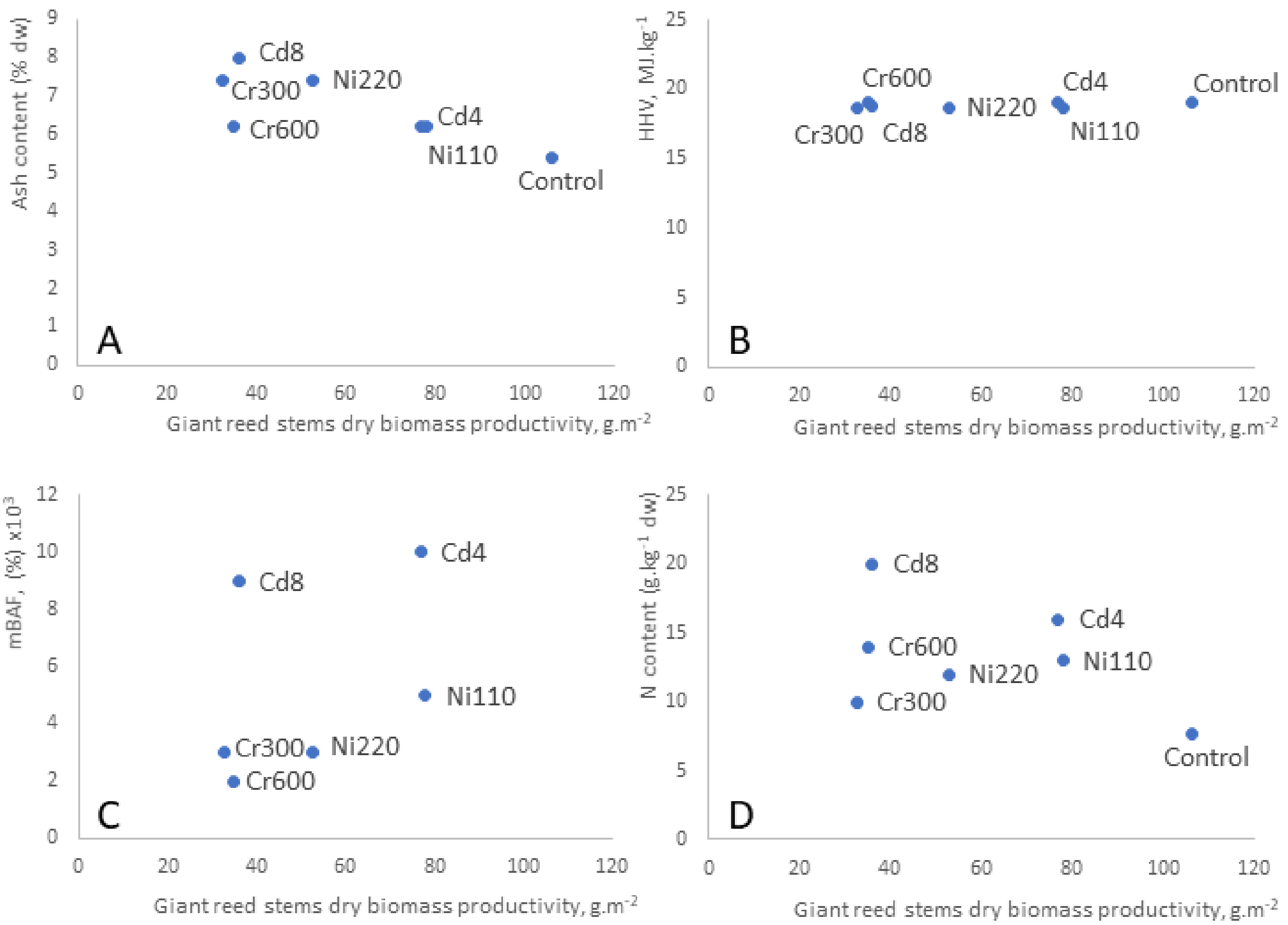

Figure 5. Global evaluation of giant reed stems energy potential when cultivated in Cd-, Cr-, and Ni-contaminated soils. Yields (g/m^2) are related with ash content (% dry weight, dw) (**A**), HHV (MJ/kg) (**B**), % mBAF (modified bioaccumulation factor) (**C**), and nitrogen content (g.kg^{-1} dry weight, dw) (**D**). Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1} dry matter; Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1} dry matter; Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1} dry matter.

4. Conclusions

Results demonstrate giant reed and switchgrass' energetic potential, even when cultivated in heavy metals contaminated soils. Despite the decrease in productivity due to the contamination of the pots, both crops showed a certain accumulation potential for the heavy metals, especially in the roots, evidencing its potential for phytoremediation through the stabilization of the contaminants. An exception was observed in the case of switchgrass with Cr contamination when this crop did not produce biomass in those pots.

The valorization of the contaminated biomass is vital to make phytoremediation processes economically feasible and to provide low ILUC risk crops with an added value. In this way, switchgrass and giant reed showed to be promising feedstocks for bioproducts and bioenergy. However, their variability in chemical composition due to the contamination of the soil creates challenges. Indeed, to obtain a product with sufficient quality and to achieve uniform conversion efficiencies, it is mandatory to understand the range of variation and the factors that influence the biomass characteristics. This knowledge will serve to improve the processing of the biomass. Results obtained indicate that giant reed ash and nitrogen content were both affected by the level of contamination: the higher the level of contamination, the higher the ash and N content. In the case of switchgrass, nitrogen content was also affected by the contamination, but not the ash content. However, contamination did not affect the HHV of both crops, which shows an opportunity for its valorization. This study brought to light more knowledge as to the interactions between the type of soil contamination and the yields and biomass quality of switchgrass and giant reed. However, more studies are needed, including research as to different contaminants, different types of soil, and different crops, in order to have a broader view on the options via which to obtain energy from energy crops cultivated in soils contaminated with heavy metals.

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