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Arundo donax L. Biomass Production in a Polluted Area: Effects of Two Harvest Timings on Heavy Metals Uptake

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Abstract: Within the framework of energy biomass production, *Arundo donax* L. is very promising for its capability to grow on marginal lands with high yields. This potential can be realized in unused polluted areas where the energy production can be coupled with phytoremediation, and harvested biomass represents a resource and a means to remove contaminants from the soil. Two main processes are considered to evaluate *A. donax* L. biomass as an energy crop, determined by the timing of harvest: anaerobic digestion with fresh biomass before winter and combustion (e.g., pyrolysis and gasification) of dry canes in late winter. The aim of this work was to evaluate the use of *A. donax* L. in an area polluted by heavy metals for phytoextraction and energy production at two different harvest times (October and February). For that purpose, we established in polluted area in northern Italy (Caffaro area, Brescia) an experimental field of *A. donax*, and included switchgrass (*Panicum virgatum* L.) and mixed meadow species as controls. The results obtained by ICP-MS analysis performed on harvested biomasses highlighted a differential uptake of heavy metals depending on harvest time. In particular, considering the yield in the third year, A. donax was able to remove from the soil 3.87 kg ha^{-1} of Zn, 2.09 kg ha^{-1} of Cu and 0.007 kg ha^{-1} of Cd when harvested in October. Production of *A. donax* L. for anaerobic digestion or combustion in polluted areas represents a potential solution for both energy production and phytoextraction of heavy metals, in particular Cu, Zn and Cd.

Keywords: *Arundo donax*; switchgrass; heavy metals; phytoremediation; harvest time

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1. Introduction

The ability of giant reed (*Arundo donax* L.) to grow on marginal lands means that this plant can be cultivated on soils not suitable for traditional purposes. This plant species produces large amounts of biomass in comparison with traditional energy crops [1]; the adaptability of *A. donax* L. makes this plant fitting for marginal agricultural areas unsuitable for conventional agriculture. In fact, yields up to 20 Mg DM ha^{-1} of aboveground biomass at the third year in low-fertility sandy soil have been reported, without the need for any agronomic input, such as weed control and irrigation [2].

The interest in giant reeds is growing because of its high biomass yields and low production costs, and also, for its versatility of usage. Giant reed, in fact, finds applications in the production of bioenergy by different means, such as biogas and bioethanol production, biomass combustion [1] and pyrolysis/gasification [3,4]. This is due to the high calorific value of 18.7 ± 1.2 MJ kg^{-1} that is comparable with that of other herbaceous grasses, i.e., switchgrass and miscanthus (HHV of 19.1 kg^{-1} and 18.7 MJ kg^{-1} respectively), and woody biomasses such as poplar (HHV of 19.5 MJ kg^{-1}). In a temperate climate, giant reed, miscanthus, switchgrass and poplar can produce 37–38, 15–30, 10–25 and 9–20 Mg DM ha^{-1}, respectively [5]; hence, we can deduce that giant reed, potentially, can produce about respectively 40%, 52% and 59% more energy per hectare in comparison with the other three crops.

However, even when they have similar calorific values, in general the quality of biomasses, ashes and combustion fumes of herbaceous plants, compared to tree species is significantly lower. In fact, herbaceous biomasses are much richer in N, Si, Ca, K, Na, Mg, Al, S and Cl compared to woody biomasses, leading to a substantial increase in the amount of ashes (more than 10 times), which are low melting, creating problems in direct combustion due to maintenance problems in the combustion system. Consequently, the emissions of the herbaceous biomasses' combustion are more polluting, being much richer in total dust, NOx, HCl, SO_2 and CO compared to woody plants [4,6,7]. For these reasons, the optimization of the pyrolysis/gasification process could mitigate the problems of the quality of the emissions and concentrate the heavy metals present in the biomass in the solid fraction [4,8].

Heavy metals, hydrocarbons and dioxins can affect soil ecosystems sufficiently to result in significant losses in soil quality [9,10]. As for the soil pollution problem, phosphate-containing fertilizers are contaminating agro-ecosystems, mainly with cadmium. Other heavy metal sources are refuse dumps and the applications of sewage sludge [11–13] and Bordeaux mixture (a mixture of copper sulfate and lime) within agriculture. Italy is the fifth most populous country in Europe (about 60 million citizens) and it is a highly developed country. Lombardy, in particular, is characterized by both intensive agriculture and by industries and vehicular traffic; all these activities are sources of air, water and soil pollution. In Italy there are about 15,000 potential contaminated sites. Of these, about 3400 have been declared contaminated and 18 are of national interest for a total of about 100,000 ha. About 30 billion € are needed for decontamination. From 2001 to 2012, about 3.6 billion € was mobilized. The Lombardy Region approved a resolution providing an allocation of 41 million € for the period 2013–2015. These funds are provided to municipalities, provinces and ARPA, to perform new remediation or to supplement those works already in progress, allowing work to continue and to complete interventions on at least 16 contaminated sites [13].

Of course, plants cultivated on contaminated soil absorb different metals, some of which are known to be toxic at low concentrations: because plants constitute the basis of the food chain, the quality of the soils is of primary importance [14].

Metals cannot be degraded, and cleanup generally requires their removal from the soil by "dig and dump" which is expensive and inhibits soil fertility, causing negative impacts on the ecosystem. Despite its potential, phytoremediation is yet to become a commercially viable technology because of the cost and the time needed. However, it would be one of the best approaches to remove contamination from the soil, without destroying the soil structure and fertility. In the phytoremediation process, major mechanisms involve the roots and rhizosphere (filtration, degradation) and the whole plant (degradation, volatilization, stabilization, extraction). Hence, the phytoremediation process could be the best technique for the remediation of widespread polluted areas, in particular where metals are present at relatively low concentrations in the surface layers of the soil [15,16]. The main approaches proposed for phytoremediation are "chelate-assisted phytoextraction" and "continuous phytoextraction." In the first case the mobility and the uptake of metals in soil are increased by artificial chelates added to soil, in the second case the natural ability of plants to absorb metals is used [17]. Hyperaccumulator plant species could represent a winning solution if they did not still have various limits to their use, such as low biomass production and shallow root systems [18,19]. To overcome these limits we will have to create plants capable of hyperaccumulating metals and at the same time producing a large amount of biomass; the removed heavy metals could be recycled from the harvested contaminated plant biomass [20]. Hence, phytoextraction potential is mainly influenced by two factors:

(i) element concentration in aboveground biomass and (ii) yield of aboveground dry biomass. Several pilot experiments have been performed in recent years using annual and perennial woody plants, showing the potential of this technique [21]. Producing energy from biomass increases considerably the sustainability of phytoremediation action [22] and generates a positive impact due to fossil fuel substitution. Remediation techniques that use soil–plant systems cannot be limited to one decontamination mechanism. Many processes occur simultaneously in every in situ application. In a field trial, different strategies can be adopted to obtain the desired effect on each soil pollutant. Degradation and volatilization through plant stomata of arsenic [23] and cadmium phytoextraction and adsorption on raw leaves [24], for example, can be maximized with the same crop management choices. Plants can have morphological and physiological responses to organic pollutants. Phenantrene, for example, shows high toxicity in maize and leads to water stress symptoms, with higher calcium, phosphorus, magnesium and zinc contents in leaves [25]. Thus, the interfering of some pollutants with transpiration and micro-element balance needs to be taken into account.

Until now, phytoremediation has been limited by the long period of time plants require to reduce contaminant levels [26,27]. Optimal plants for phytoremediation should have the following characteristics: (i) high biomass production harvested per year with economic value; (ii) extensive root systems; (iii) tolerance and accumulations of heavy metals in harvestable biomass.

This description resembles the characteristics of giant reed, which is a perennial rhizomatous grass, widely diffused in subtropical and temperate regions; due to its great adaptability and well-documented tolerance to heavy metals contamination, it is considered as one of the most promising energy crops [28].

This species can grow in polluted soil where the concentration of each of Cd^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} is 100 mg kg $^{-1}$, and that of Cr^{6+} is 50 mg/kg [29]. The characteristics of high biomass production and the exuberant root system of *A. donax* L. suggest that it has great potential in remediation of polluted soils.

Cultivating *A. donax* L. on contaminated lands can contribute to the availability of higher-quality land and limit the food vs. plant-based feedstock controversy. Persistent occupation of the soil by inedible plants is a form of pollution mitigation, an ecological passage and a pool for biodiversity for any urban or suburban area. A positive impact on the environment is generated even at an early stage and in sites where urbanization has left scarcely any solutions for environmental recovery. The use of *A. donax* L. for phytoremediation is well documented in removing As, Cd, Cr, Fe, Hg, Mn, Ni, Pb and Zn and for treating urban wastewater, aqueous solutions from industrial processes and in general wastewater containing organic compounds [1]. Some ex situ experimentation found that there was slightly to no loss in biomass production of *A. donax* L. on polluted soils, and it showed its capability to extract heavy metals in response to their presence in the soil [1]. However, limited growth conditions in ex situ (e.g., pot) experimentation can limit the significance of these studies [30]. For this reason, we established an experimental field of *A. donax* L., switchgrass (*Panicum virgatum* L.) and mixed meadow species in an area polluted by heavy metals. The polluted site chosen for this work was located in the Caffaro area (Brescia, North Italy), with an area of 12.5 hectares. This site was previously characterized (by ERSAF Brescia): the concentration values of different heavy metals and dioxins in the surface layers of the soil (0–40 cm) exceed the values shown in Legislative Decree number 152 of 2006 [27] concerning the remediation of contaminated sites, column “A” (soil for residential/public green) and for some items, column “B” (soil for industrial use), (Italy, 2006). The experiment was conducted for 3 years, with the objective of: (a) comparison of the biomass yield; (b) assessing the capacity to accumulate heavy metals in the aboveground biomass; and (c) assessing the best harvesting period to maximize the metals’ concentrations in the biomass.

2. Materials and Methods

2.1. Plant Material

The three-year experiment described in this study was performed using the selected Ad20 clone from the Italian *Arundo donax* L. (Ad) collection located in Landriano (PV, Italy, 45°18′ N, 9°15′ E); sown switchgrass (Pv) var. Alamo; and as control, the mixed meadows (MM) species already present in the area. The *A. donax* L. clone Ad20 represents a promising genotype for large scale cultivation, due to its production potential and efficient propagation [31–33]. The control MM was constituted by the predominant species *Medicago sativa* and *Chenopodium album*. No management of the field had been done for the previous 5 years.

2.2. Field Establishment

The experiments described in this study were carried out in a field within the Caffaro area (N 45.5358, E 10.1841). The soil characteristics at the experimental site are reported in Table 1, which includes the contents of 12 heavy metal pollutants resulting from ICP-MS analysis, as described in Section 2.4.

Table 1. Soil characteristics at the experimental site 0–30 cm (March 2017) and soil thresholds for residential/recreational and commercial or industrial use, by Italian norms concerning the remediation of contaminated soils [34].

Parameter	Value	Residential-Recreational Land Use Threshold	Commercial or Industrial Land Use Threshold
Sand (2–0.05 mm) (%)	38.49		
Silt (0.05–0.002 mm) (%)	49.84		
Clay (<0.002 mm) (%)	11.66		
pH	7.83		
Organic matter (Walkley–Black) (%)	0.14		
Total nitrogen (Kjeldahl) (g kg^{-1})	1.65		
Cation Exchange Capacity (meq 100 g^{-1})	24.77		
Al (g kg^{-1})	21.40 ± 0.62	- [1]	-
Cr (mg kg^{-1})	36.04 ± 8.18	150	800
Mn (mg kg^{-1})	805.64 ± 69.73	-	-
Fe (g kg^{-1})	23.05 ± 1.14	-	-
Co (mg kg^{-1})	12.97 ± 0.52	20	250
Ni (mg kg^{-1})	29.99 ± 3.10	120	500
Cu (mg kg^{-1})	89.38 ± 25.58	120	600
Zn (mg kg^{-1})	183.85 ± 35.26	150	1500
As (mg kg^{-1})	32.41 ± 7.09	20	50
Mo (mg kg^{-1})	0.52 ± 0.12	-	-
Cd (mg kg^{-1})	0.39 ± 0.09	2	15
Pb (mg kg^{-1})	128.25 ± 49.03	100	1000

[1] Non-regulated values in soils.

The field was established by transplanting *A. donax* plantlets with a density of 2500 plants ha^{-1} or switchgrass with a density of 10,000 plants ha^{-1}. The experimental design was a randomized block with three replications of 100 m^2. Weed competition was avoided by operating on the surrounding area with a mulching plastic film during the first year and by mowing operations in the second and third years.

Plantlets were irrigated as needed after transplantation to avoid water scarcity in the first year. During the subsequent two years no irrigation, no fertilization and no chemical treatments were done. Every February, after the sampling, the plots were cut down and the aboveground biomass removed.

2.3. Biomass Sampling

From each block, biomass coming from 16 m^2 was harvested and weighed in October or in February for three harvest seasons during the years 2016–2020. After shredding, the dry matter (DM) of biomass was obtained by inserting the samples in pre-weighed aluminum bags which were dried in a forced-air oven at 80 °C for 72 h. All dried plants were ground with a laboratory mill to 0.5 mm (Cyclone Sample Mill, Model 3010-019, pbi International, Milan, Italy).

2.4. ICP-MS Analysis

Each sample was divided into three subsamples for technical repetitions. The analyses were performed on digested 300 mg of dry matter (65% HNO_3) by a microwave digester system (Anton Paar Multiwave 3000, Austria). Diluted samples were measured by ICP-MS (Varian 820 ICPMS, Agilent, Santa Clara, CA, USA) as reported in [35].

2.5. Statistical Analysis

Species (S), year (Y) and harvest time (HT) factors were considered as independent variables, while the three blocks for each treatment represented the random factor. Statistical significance of effects was examined by IBM SPSS version 20 software [36]. The tests used a confidence interval of 95% and the normal distribution, and homoscedasticity of treatments was assessed by, respectively, the Shapiro–Wilk test under command EXPLORE and the Levene test. To discriminate differences in biomass yield among treatments, a univariate approach was applied, with the command UNIANOVA under satisfied requisites of the analysis of variance. For each block, results of ICP-MS on the three subsamples were aggregated to their mean value. A PCA analysis of heavy metals' biomass concentrations as components was conducted on standardized values for each treatment with PAST version 3.12 software [37]. A multivariate approach to analyze heavy metals' biomass contents was applied by GLM command on SPSS software, with re-sampling of cases by wild residual BOOTSTRAP command with 1100 repetitions, as some treatments did not satisfy the requisites of analysis of variance. Post hoc analysis was performed by Tukey's test and pairwise comparisons were performed by EMMEANS subcommand, using Sidak as the adjustment method.

3. Results

3.1. Biomass Yield

Considering the production of biomass, the main effects of the factors species (S), year (Y) and their interaction were significant ($p < 0.05$), but no effect was found for the factor harvest time (HT). The mixed meadow (MM) showed, as expected, homogeneous production during the three years of experimentation, with an estimated mean of 13.23 ± 1.33 tonnes DM ha^{-1} at each harvest. During the first year, *A. donax* L. (Ad) and switchgrass (Pv) produced less biomass compared to controls, with a mean production of 7.67 ± 1.66 tonnes DM ha^{-1}. In the second year, the biomass yields of both Ad and Pv were statistically larger than the control MM ($p < 0.05$), as can be observed in Figure 1.

Pv increased its production in the second year with no significant difference compared to the third year, with an estimated biomass yield of 18.35 ± 1.58 tons DM ha^{-1}. Ad had an increase in biomass production in both the second and third years, with biomass yields of, respectively, 27.90 ± 2.81 and 33.29 ± 2.96 tons DM ha^{-1}. The homogeneous subgroups are shown in Figure 2.

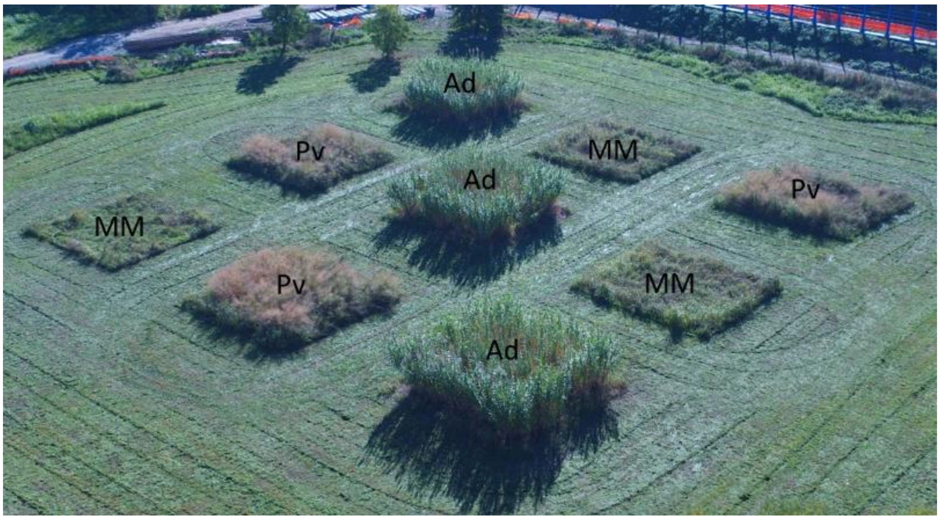

Figure 1. Experimental field photographed from the air during the period of this study. The letters represent the different species used: *A. donax* (Ad); switchgrass (Pv) and mixed meadow (MM). Each block has the dimensions of 10 m × 10 m with a distance of 10 m between the plots. The grass between the plots was regularly mown, to avoid growth of surrounding weeds.

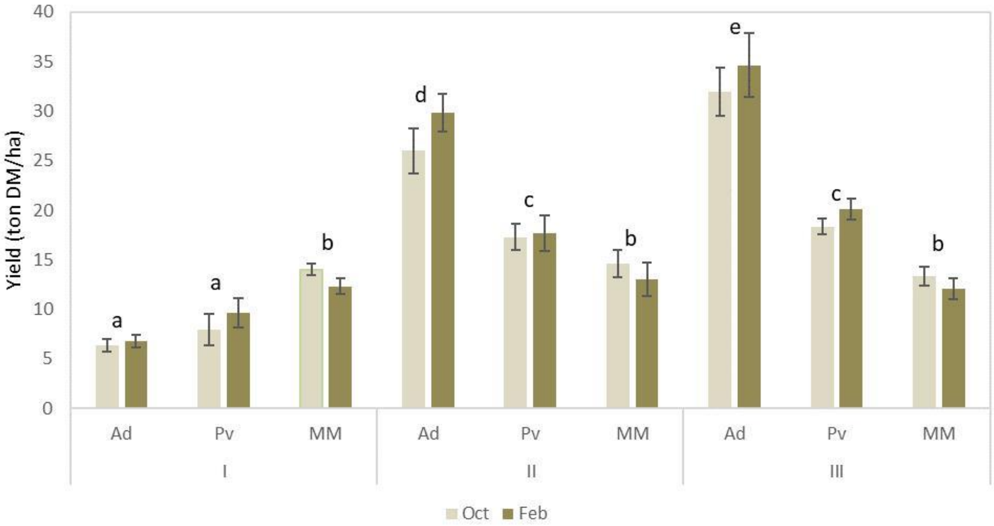

Figure 2. Estimated biomass yield of the treatments, each column representing each harvest operation under the different combination of year (I, II and III), species (Arundo—Ad, switchgrass—Pv and mixed meadows—MM) and harvest times (October and February). Error bars represent SD and letters a, b and c label statistically homogeneous subgroups ($p < 0.05$).

3.2. *Heavy Metals in Harvested Biomass*

ICP-MS results for the three subsamples (randomized blocks) for each treatment regarding Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd and Pb showed about 1% of outlier data. The normality of distribution of the different combinations of factors was confirmed for 95% of treatments, but homoscedasticity was not confirmed by the vast majority of samples ($\alpha = 0.05$). Table 2 summarizes the mean values and standard deviations for each heavy metal among the different samples.

Table 2. ICP-MS analysis on 12 heavy metals in the harvested biomass of samples by Year (Y), Species (S) and Harvest Time (HT) expressed as mg/kg of DM. Each value is followed by its SD.

Y	S	HT	Pb	Cd	Mo	As	Zn	Cu	Ni	Co	Fe	Mn	Cr	Al
I	Ad	October	0.43 ± 0.1	0.06 ± 0.01	1.39 ± 0.22	0.06 ± 0.03	76.05 ± 23.24	8.66 ± 0.31	0.02 ± 0	0.02 ± 0.01	56.21 ± 7.54	62.47 ± 8.37	1.56 ± 0.38	12.08 ± 2.33
		February	0.61 ± 0.06	0.14 ± 0.07	2.02 ± 0.45	0.05 ± 0.01	39.46 ± 10.91	8.86 ± 0.93	0.5 ± 0.7	0.15 ± 0.01	90 ± 6.73	93.91 ± 20.94	4.96 ± 0.85	19.26 ± 11.6
	Pv	October	0.31 ± 0.06	0.04 ± 0.01	0.99 ± 0.64	0.04 ± 0.03	24 ± 6.23	4.44 ± 1.12	0.02 ± 0.01	0.02 ± 0.02	38.15 ± 9.61	16.19 ± 7.4	2.03 ± 0.08	11.3 ± 8.15
		February	1.76 ± 0.56	0.02 ± 0.01	2.21 ± 1.94	0.03 ± 0.01	52.17 ± 13.61	10.96 ± 2.85	1.87 ± 1.13	0.19 ± 0.04	180.74 ± 58.9	19.37 ± 6.22	8.26 ± 3.37	65.58 ± 21.78
	M	October	0.41 ± 0.05	0.02 ± 0.01	2.72 ± 0.75	0.02 ± 0.01	47.21 ± 24.73	5.05 ± 2.07	0.02 ± 0.01	0.03 ± 0.01	35.97 ± 20.35	30.2 ± 7.46	0.88 ± 0.78	11.85 ± 14.65
		February	1.34 ± 1.19	0.03 ± 0.01	1.6 ± 1.37	0.05 ± 0.05	30.7 ± 6.51	9.52 ± 5.69	0.24. ± 0.39	0.29 ± 0.36	125.4 ± 104.8	30.57 ± 10.86	1.51 ± 1.4	48.19 ± 11.01
II	Ad	October	0.12 ± 0.06	0.02 ± 0.01	2.09 ± 0.58	0.18 ± 0.02	115.1 ± 100.7	7.4 ± 1.61	0.8 ± 0.05	0.05 ± 0.01	72.91 ± 35.59	20.68 ± 2.93	1.8 ± 0.8	15.42 ± 7.96
		February	0.35 ± 0.06	0.04 ± 0.02	1.69 ± 0.07	0.26 ± 0.18	25.65 ± 2.46	5.24 ± 0.54	0.85 ± 0.15	0.08 ± 0.02	62.76 ± 15.69	27.48 ± 3.47	2.74 ± 0.71	24.05 ± 7.78
	Pv	October	0.3 ± 0.04	0.21 ± 0.17	2.57 ± 1.53	0.2 ± 0.04	43.76 ± 13.83	5.71 ± 1.08	1.06 ± 0.53	0.05 ± 0.01	71.26 ± 6.72	16.07 ± 10.05	3.39 ± 0.76	27.17 ± 7.23
		February	0.61 ± 0.19	0.03 ± 0.01	1.95 ± 1.38	0.31 ± 0.28	39.65 ± 11.42	6.04 ± 1.18	1.14 ± 0.37	0.05 ± 0.01	88.71 ± 22.17	13.14 ± 3.01	4.38 ± 1	40.68 ± 13.73
	M	October	0.46 ± 0.08	0.05 ± 0.01	4.67 ± 2.23	0.28 ± 0.04	62.69 ± 14	8.58 ± 1.8	1.03 ± 0.36	0.07 ± 0.02	104.17 ± 10.25	28.07 ± 14.15	3.49 ± 1.47	34.79 ± 1.66
		February	0.84 ± 0.05	0.03 ± 0.02	4.49 ± 3.65	0.24 ± 0.06	88.6 ± 26.06	6.19 ± 1.5	1.13 ± 0.24	0.12 ± 0.04	148.72 ± 22.79	24.08 ± 7.88	4.24 ± 0.27	88.29 ± 24.08
III	Ad	October	0.76 ± 0.53	0.21 ± 0.05	2.22 ± 0.44	0.11 ± 0.06	121 ± 110.01	6.55 ± 1.59	0.81 ± 0.56	0.16 ± 0.02	74.07 ± 39.57	21.71 ± 4.42	1.48 ± 0.59	16.26 ± 11.2
		February	0.48 ± 0.02	0.11 ± 0.01	1.58 ± 0.24	0.07 ± 0.01	28.51 ± 2.4	4.94 ± 0.3	0.74 ± 0.05	0.1 ± 0.03	68.99 ± 16.82	30.06 ± 3.66	2.56 ± 0.93	23.85 ± 8.78
	Pv	October	0.51 ± 0.23	0.35 ± 0.18	2.21 ± 1.33	0.11 ± 0.01	45.53 ± 13.41	6.52 ± 2.27	1.39 ± 0.58	0.16 ± 0.03	85.7 ± 3.2	16.78 ± 8.38	3.5 ± 0.92	27.81 ± 5.16
		February	0.8 ± 0.32	0.09 ± 0.01	2.11 ± 1.97	0.07 ± 0.03	39.94 ± 8.14	5.72 ± 0.72	1.23 ± 0.28	0.11 ± 0.01	97.77 ± 17.19	15.11 ± 1.84	3.24 ± 0.11	35.97 ± 16.56
	M	October	0.92 ± 0.36	0.13 ± 0.06	4.05 ± 1.9	0.16 ± 0.03	67.84 ± 11.02	9.85 ± 1.57	0.89 ± 0.48	0.16 ± 0.03	125.63 ± 13.2	30.39 ± 14.22	3.24 ± 1.16	32.7 ± 1.53
		February	1.39 ± 0.71	0.13 ± 0.03	4.05 ± 2.88	0.09 ± 0.02	94.84 ± 23.84	6.43 ± 0.77	1.17 ± 0.5	0.21 ± 0.06	162.4 ± 14.39	25.69 ± 5.17	3.53 ± 0.61	88.17 ± 22.28

Considering a three-way interaction design for year (Y), species (S) and harvest time (HT) factors, 99% of combinations were statistically significant ($p < 0.05$). The analysis of variance designed with a two-way interaction design highlighted a significant effect of factor Y as main effect and/or in its interaction with S and HT, for all heavy metals considered in this experiment ($p < 0.05$), except Zn. By pairwise comparisons among Y levels on main and combined effects, significant differences were highlighted when comparing the first year with second and third years, for all heavy metals ($p < 0.05$), representing 81% of significant differences caused by this factor.

In almost all cases, metals were less accumulated in the biomasses of the first year than in the other two years, since the plants had not reached maturity.

Considering these results, the first year was excluded from the following calculations to consistently reduce type 2 errors in the analysis.

PCA analysis picked out Zn, Fe and Al as the three most relevant components explaining almost all the variance among samples in the second and third years. The results highlighted that treatments were clustering by HT and S factors, whereas Y appeared to influence only the scattering within the clusters (Figure 3).

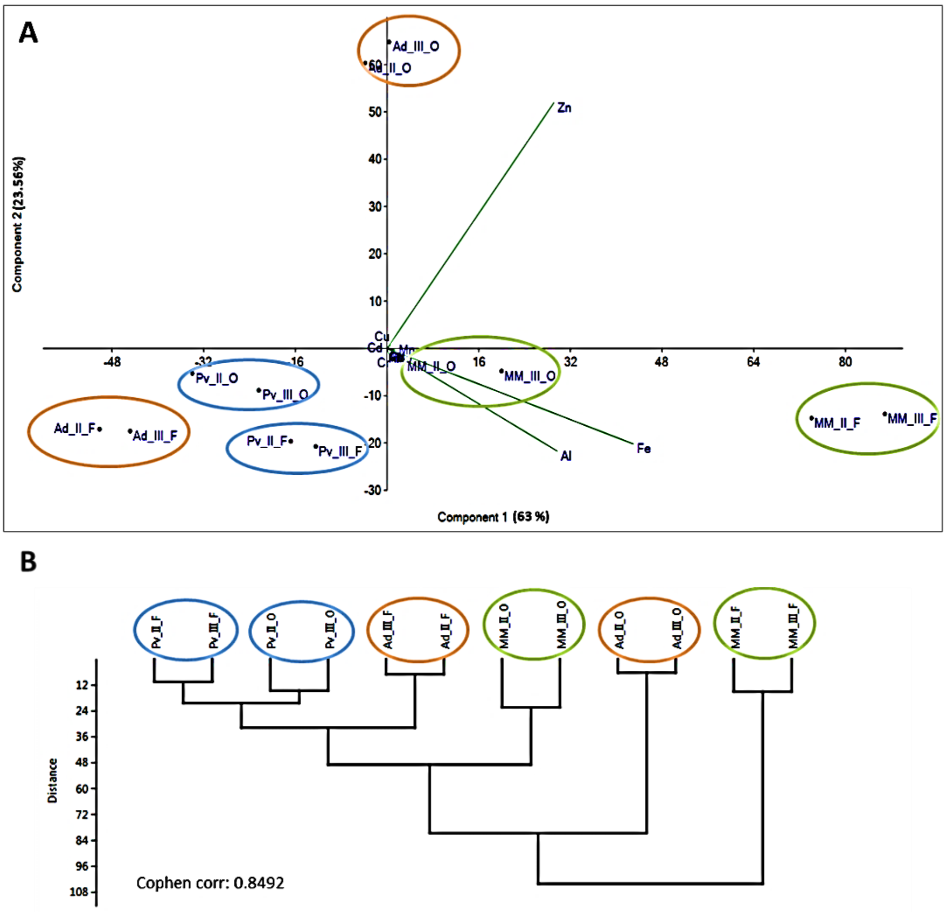

Figure 3. PCA biplot (**A**) and clustering analysis (**B**) based on the accumulation of 12 metals (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd and Pb) in the biomass of *A. donax* L. (Ad), switchgrass (Pv) and mixed meadow (MM), considering the year of cultivation, second (_II) and third (_III), and the harvest time (October: _O and February: _F). In PCA analysis the first two axes (PC1 and PC2) explain 86.56% of the total variance.

For the last two years of the experiments, the factor Y maintained an effect on heavy metals' uptake only for Co, As, Cd and Pb—elements sharing low accumulation in the harvested biomass, rarely exceeding 1 mg/kg^{-1} (Table 2). By multivariate analysis of variances, different effects were highlighted in the second and third years, with species (S) and harvest time (HT) factors and/or their interaction being significant ($p < 0.05$) for all metals excluding As and Zn. Samples' contents of the 12 heavy metals are plotted in Figure 4.

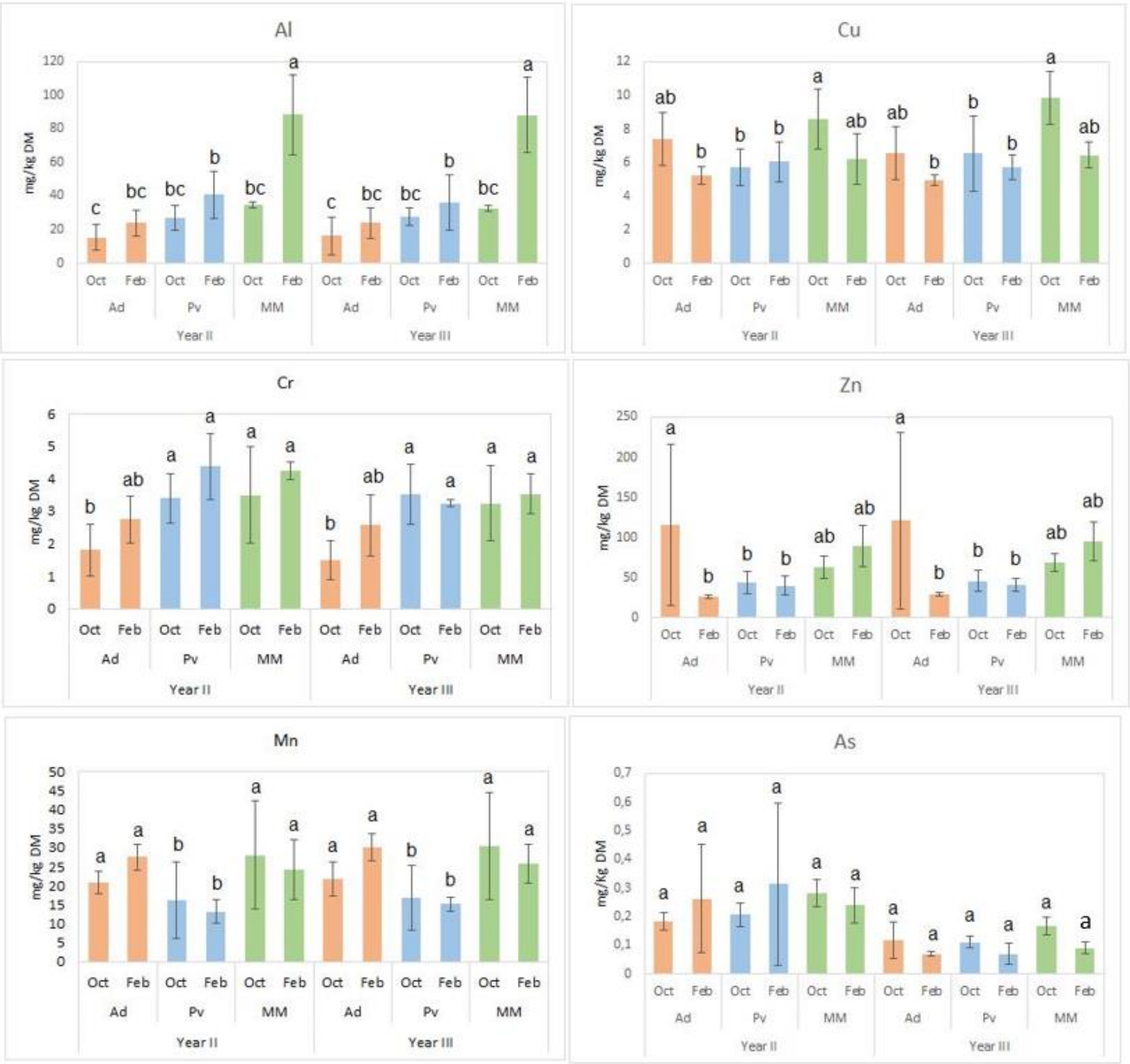

Figure 4. *Cont.*

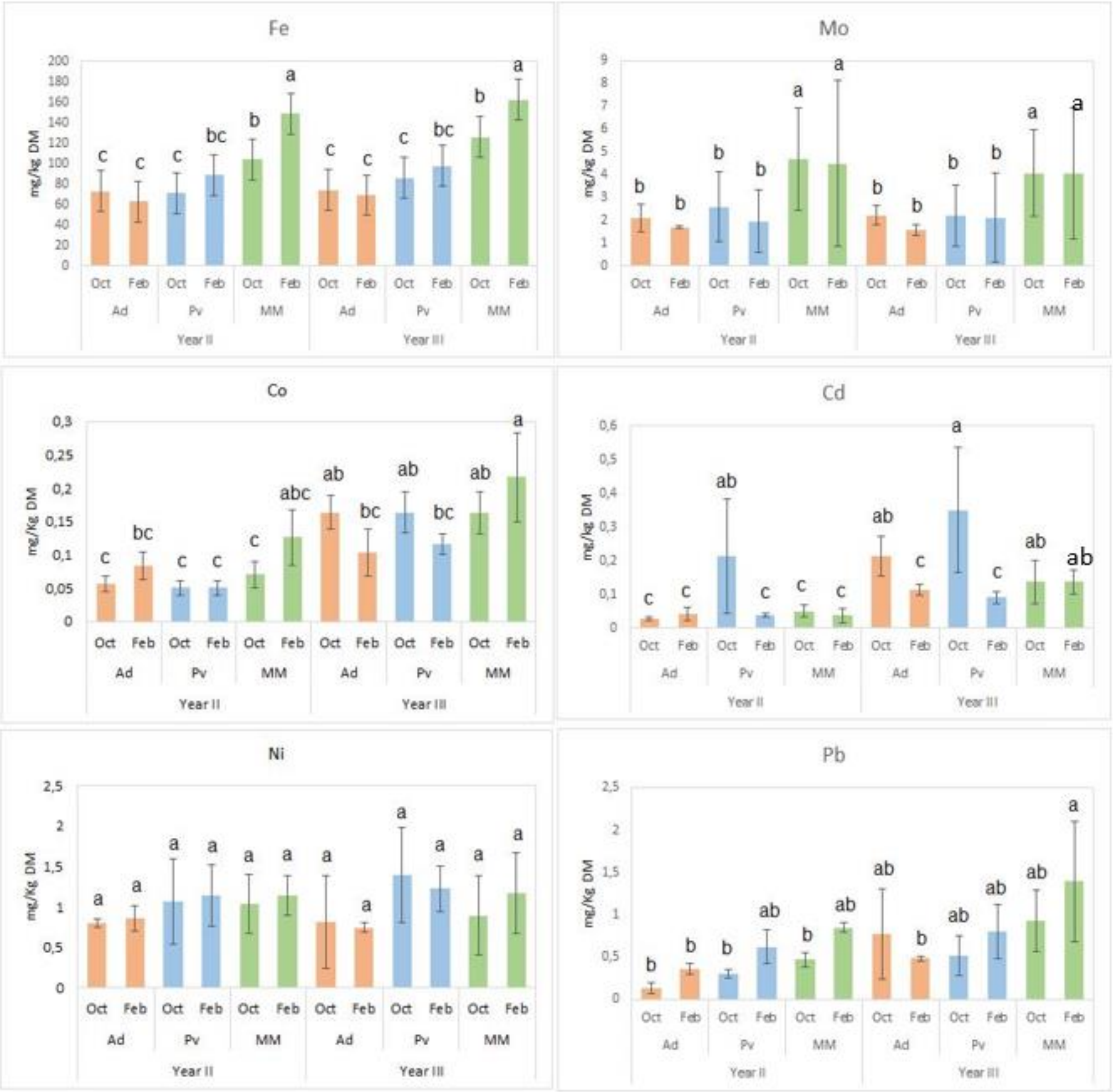

Figure 4. Accumulation of heavy metals in the harvested biomasses of the second and third years, separated into subgroups by Tukey HSD test. For Mn and Mo, groups were separated by species (S); for Al, Cr, Fe, Cu and Zn homogeneous groups were distinguished by species (S) and harvest time (HT); for Co, Cd and Pb, groups considered S, HT and year (Y) factors. Bars represent SD.

3.3. Species and Harvest Time Effects

The species factor (S) effect was significant (p <0.05) on harvested biomass accumulation for all heavy metals except Ni, Zn and As. *A. donax* L. (Ad) and switchgrass (Pv) had a similar accumulations of some heavy metals in the harvested biomasses, with Al, Fe, Cu, Mo and Pb contents being lower than those of mixed meadows (MM). Considering Cr, Mn, Ad and Pv, they were, respectively, lower than in MM. The effect of the harvest time factor (HT) was significant ($p < 0.05$) for Al, Cr, Fe, Cu, Cd and Pb harvested biomass uptake. The trend for Al, Cr and Fe was for a higher content of heavy metal from the February harvest, whereas Cu, Cd and Zn showed an opposite dynamic, with more accumulation for the October harvest. The two factors species (S) and harvest time (HT) had significant interactions ($p < 0.05$) for Al, Fe, Co, Zn and Cd, highlighting specific patterns of heavy metal accumulation. In particular, the control MM harvested in October accumulated more than two-fold the Al in comparison with other treatments. The same sampling was also favorable for Fe accumulation. For *A. donax* L., (Ad) accumulation of Zn was largely affected by HT, showing the highest and lowest results among samples for, respectively, October and February harvests. A similar effect of HT was highlighted for switchgrass (Pv) in Cd accumulation, with a more favorable HT in October. Considering the accumulation of Co, effects of year (Y) with the factors S and HT were significant: the control MM kept the same trend during the second and third years, whereas both Ad and Pv showed an overwinter increase of this element during the second year, but an overwinter reduction during the third year.

3.4. Uptake Potential

In Table 3 we report the amounts of heavy metals, expressed as kg ha^{-1}, that could be removed by phytoextraction in this polluted area, considering the third-year yield. In almost all cases *A. donax*, due to its high yield, was able to phytoextract higher amounts of heavy metals compared to switchgrass and mixed meadow. Furthermore, apart from Mn, Cr and Al, the best harvest time to maximize the phytoextraction process turned out to be October, when the biomass harvested could be used in anaerobic digestion.

Table 3. Heavy metal uptake in the Caffaro area considering the yield from the third year of the experiment. Values are listed for *A. donax* L. (Ad), switchgrass (Pv) and mixed meadows (MM), for October (Oct) and February (Feb) harvests. Higher values are shown on a gray background.

Heavy Metal	**Uptake in Third Year (kg ha^{-1})**					
	Ad Oct	**Ad Feb**	**Pv Oct**	**Pv Feb**	**MM Oct**	**MM Feb**
Zn	3.87	0.99	0.84	0.80	0.91	1.14
Fe	2.37	2.39	1.57	1.96	1.68	1.96
Cu	2.09	1.7	0.12	0.11	0.13	0.08
Mn	0.69	1.04	0.31	0.30	0.41	0.31
Al	0.52	0.83	0.51	0.72	0.44	1.06
Mo	0.07	0.05	0.04	0.04	0.05	0.05
Cr	0.05	0.09	0.06	0.07	0.04	0.04
Ni	0.03	0.03	0.03	0.02	0.01	0.01
Pb	0.02	0.02	0.01	0.02	0.01	0.02
Cd	0.007	0.004	0.006	0.002	0.002	0.002
Co	0.005	0.004	0.003	0.002	0.002	0.003
As	0.004	0.002	0.002	0.001	0.002	0.001

4. Discussion

Giant reed, considered for many years only as a weed, is now considered as an energy crop that can be used in marginal areas that cannot be used by conventional agriculture [2,38].

Due to anthropic activities, marginal lands are increasing worldwide mainly because of salinization caused by irrigation [39] and heavy metal contamination caused by industrial activities [40]. This could mean more opportunities to cultivate energy crops which are environmentally friendly, such as giant reed crops, in the near future.

The consideration of novel strategies for the sustainable use of marginal lands in Mediterranean areas was the aim of the European project, OPTIMA (Optimization of Perennial Grasses for Biomass Production in the Mediterranean Area) [41]. In this framework, considering four perennial species, cardoon, giant reed, miscanthus and switchgrass, the OPTIMA project highlighted giant reed when compared to the other energy crops [42,43].

Furthermore, deep-rooted perennial grasses such as giant reed (*Arundo donax* L.) and switchgrass (*Panicum virgatum* L.) can provide a contribution to the reduction of greenhouse-gas emissions [44,45], producing biomass to displace fossil fuels [46] and for the phytoremediation of polluted soils.

In our study, *A. donax*, switchgrass and mixed meadow biomasses produced in a polluted area in northern Italy were compared for two harvest times (October and February) in a three year experiment, with the aim of assessing the capacity of phytoextraction of the heavy metals and the best outcome for energy production (mainly anaerobic digestion and combustion) (Figure 1). This research started from the results of project LUCAS's topsoil survey, which built a consistent spatial database of polluted soil across Europe. In this project it was estimated that 137,000 km^2 needs local assessment and eventual remediation action [47].

Giant reed biomass has found wide applications in energy production; it can be used in combustion, gasification and pyrolysis and to produce biogas [1]. In fact, when harvested in autumn its biomass can be used in anaerobic digestion in substitution/partial integration with the traditional energy crops [48–50], and the biomass harvested in winter can be used for the combustion process, due to its high heating value of about 18.7 MJ kg^{-1} [1].

The estimated yields for *Arundo* and switchgrass in the third year, obtained in this work, reported in Figure 2, (respectively, about 33 and 19 tons ha^{-1}), substantially confirm the data present in the literature where *A. donax* in the Mediterranean basin is the most productive energy crop [1,51–53]. We decided not to consider the data obtained in the first year of experimentation—although it is reported in Table 2—given that in the first year of planting both *Arundo* and switchgrass were not in full production and had not fully developed either their rhizomes or the root systems. This decision was supported by pairwise comparisons among Y levels on main and combined effects, where significant differences were observed when comparing the first year with second and third years, for all heavy metals accumulated. In fact, when considering the data reported in Figure 3, concerning the biplot PCA and clustering analysis obtained for the 12 heavy metals considered in this study, the same accumulation pattern was found in the second and third years.

The results shown in Table 2 and Figure 4, regarding the accumulation of heavy metals in the biomasses of the three different species, are in agreement with previous work reporting the bioaccumulation factor (BAF = C plant/C soil) considering only the biomass harvested of Ad [54], Pv [55] and MM [56].

The highest value of phytoextraction was found for Zn (Figure 4). Zn is an essential micronutrient for plant growth; hence, it is not surprising that it was present in the harvested biomass in higher concentrations in comparison to the other heavy metals such as Cu, Co, Fe, Ni, Mn and Mo that can also be removed from soils via phytoextraction [57]. For the heavy metals that lack a known biological function (Al, Cr, Cd and Pb), but represent an important environmental issue, significant uptake has been reported [58–60]. The metals accumulated in plant biomass reflect the metal contents present in the soil. Each species has its own accumulation profile (we could say a chemical barcoding or chemotype) as shown in the PCA (Figure 3), where the analyses of the various biomasses cluster by species (Ad, Pv and MM), year (II and III) and harvest period (October and February). One of the main factors that can explain the differences observed among different species lies in the different capacities that plants have to release organic compounds (e.g., chelators) via root apparatus capable of modifying the rhizosphere and the pH of the soil. Of course, these chemical changes affecting the soil around the root system increase the solubility of metals [61]. Another aspect to take into consideration lies in the different physiology of the plants under study: Ad and Pv are monocotyledons, whereas MM consisted mainly of two dicotyledonous species (*Medicago sativa* and *Chenopodium album*). Furthermore, Ad and Pv are rhizomatous plants, but the rhizomatous apparatus of arundo is much larger, and Pv is able to produce seeds, as is MM [62]. Hence, by taking together all these different characteristics, we can hypothesize, starting from the results reported in Figure 3, that: (i) In the case of Ad, the difference in the metals accumulated between the biomass collected in February and October can be explained by the translocation of the nutrients present in the epigeal part of the plant towards the rhizomes (statistically significant difference in the case of Zn, Figure 4). (ii) In the case of Pv there are no major differences between the two types of biomass (harvested in October and February) probably due to the small rhizomatous apparatus, unable to translocate large quantities of nutrients. However, one statistically significant difference was observed in the case of Cd, of which more wasaccumulated in the biomass collected in October (Figure 4). In this case we can hypothesize that the Cd accumulated in the seeds still present on the plant in October was lost in February where the seeds would have fallen to the ground. The fact is well known that Cd is accumulated in plant seeds, representing a big issue for agriculture conducted on soils polluted by this metal [63]. (iii) For what concerns MM, the explanation regarding the differences observed for the metals accumulated in the two different harvest times could be more complex since it is a polyphite crop. However, the biggest difference was observed regarding Al (difference statistically significant reported in Figure 4). Al toxicity is one of the major limiting factors for crop production on arable lands [64].We can hypothesize that the higher level of Al observed in the biomass harvested in February may have been due to accumulation in the plant cell wall of the tiny stems present in MM; in fact, Al is able to displace Ca^{2+} in the cell wall [65].

Table 3 shows the amounts of heavy metals removed by phytoextraction in this polluted area, considering the third-year yield. Due to the higher yield of *A. donax* in almost all cases (Zn, Fe, Cu, Mn, Mo, Cr, Ni, Pb, Cd, Co and As) the utilization of this species allowed the removal of the greatest quantity of heavy metals compared to the crops Pv and MM. Only in the case of Al did the MM record a better value (1.06 vs. 0.83 k/ha^{-1}). Al toxicity is one of the major limitations for crop yields on acid soils, which occur in up to 30–40% of the arable lands of the world: Al is toxic to plants such as maize, barley and wheat, when solubilized into the soil solution at acidic pH values [66]. However, the difference in the Al removal between MM and Ad is not so great (0.83 vs. 1.06 kg ha^{-1} $year^{-1}$) as to justify the preferential use of MM, with which the biomass produced would be difficult to manage and not usable for producing energy. Furthermore, apart from Al, Mn and Cr, the better harvest time to maximize the phytoextraction of Zn, Fe, Cu, Mo, Ni, Pb, Cd, Co and As was in October, when the biomass harvested could be used in anaerobic digestion, a well established technology in Lombardy.

Animal production, in particular pig farms, represents a possible source of heavy metals. In fact, the major input in pig livestock is represented by feed, which should be controlled in order to prevent the excessive spread of heavy metals into the environment [35,67,68]. On the other hand, minerals such as Co, Cu, Fe, Mn, Mo and Zn are important cofactors for various enzymatic activities and consequently should be used to supplement animals' diet in accordance with the authorized levels [69].

Furthermore, Zn in particular is usually used to guarantee livestock productivity by controlling bacterial pathogen infections [70]. After the antibiotics ban in 2006 in Europe [58], there has been an increased use of high dosages of zinc oxide (ZnO). However, excessive exposure with higher concentrations of Zn has been linked to environmental issues; hence. the EU has now banned the inclusion of pharmacological levels of ZnO after 2022 [71].

5. Conclusions

In this work we reported the results concerning three years of trials regarding the cultivation of *A. donax*, switchgrass and mixed meadow in the polluted Caffaro area. The results obtained showed that A. donax is the best choice for phytoextraction and energy production due to the higher yield. In particular, considering the uptake of heavy metals in the third year of the experiment, *A. donax* is able to accumulate in its harvested biomass considerable amounts of Zn and Cu (3.87 and 2.09 kg/ha respectively). Hence, this species could become an excellent solution for phytoremediation of soils contaminated by heavy metals [72] while at the same time producing energy. Considering the serious problems within northern Italy, concerning in particular Cu, used as an anti-cryptogrammic, Zn, used extensively in pig farms and Cd, introduced with phosphate-based fertilizers, this crop could be strategic for the sustainability of agricultural supply chains.

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