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The Suitability of Short Rotation Coppice Crops for Phytoremediation of Urban Soils

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Abstract: This experiment was aimed at verifying the usefulness of phytoremediation using Short Rotation Coppice (SRC) in an urban Zn-contaminated site. Besides elemental uptake and reclamation, the SRC method was applied to evaluate the additional benefits of a green infrastructure. Nine different plants with rapid growth and large biomass production were selected: three *Populus* clones, three *Salix* hybrids, and three *Robinia* genotypes. Annual and biennial coppicing were evaluated. Poplar clones were more productive using annual coppicing, while *Salix* and *Robinia* produced higher biomass in blocks not coppiced. *Poplar* had the highest phytoextraction rate during the second year, with 1077 g/ha. *Salix* clones S1 and S3 extracted similar quantities using biennial coppicing. After two years, the bioavailable fraction of Zn decreased significantly using all species, from the 26% decrease of *Robinia* to the 36% decrease of *Salix*. The short rotation coppice method proved to be useful in an urban context, for both landscape and limiting the access to the contaminated area. Improving the biomass yield through the phytomanagement options (fertilization, irrigation, coppicing, etc.) could make SRC phytoremediation an economic and effective solution to manage urban contaminated areas, coupling the added values of biomass production to the landscape benefits.

Keywords: Short Rotation Forestry; potentially toxic elements; metals; bioavailability; urban contamination

1. Introduction

Environmental contamination from potentially toxic elements (PTE) is one of the most important issues at global level, especially in urban areas, as they are ubiquitous contaminants and could represent a threat to the health of the citizens [1–3]. In urban areas, different contamination sources coexist, with both site-related (point) and non-site (diffuse) sources, such as dust deposition, as well as traffic and parent material [4].

Current remediation methods, including excavation and transport to landfill sites, chemical and thermal treatments, incineration, and vitrification are the most frequently applied [5,6], although they have high operational costs and, potentially, a higher social and environmental impact in urban areas, for example negatively affecting the biological fertility of the soil for a new use [7]. In addition, these methods are suitable for point pollution but not for diffuse contamination, especially over large areas.

In the past decade, alternative techniques have been of increasing interest in an environmental sustainability perspective. In addition, there is public awareness on sustainable remediation together with the notion that soil is a non-renewable resource.

Bioremediation techniques, mediated by biological agents such as microorganisms or plants, encompass a number of green and sustainable remediation options. Among these, phytoremediation has been studied as both an environmentally friendly and a cost-effective practice, in particular in areas where pollutants occur at relatively low concentrations [8,9]. These areas are the most frequently found in cities due mainly to historic contamination from diffuse sources [10].

Moreover, in urban and suburban sites, soils can undergo a lower pressure for use: in fact, areas allocated to public green would have no alternative uses and could be considered stable in the short and medium term. These two aspects, which are relatively unique to urban and suburban areas, make phytoremediation a particularly attractive technique [11].

In urban areas, the soils provide also ecosystem functions, e.g., as barrier protecting water bodies from contamination, and provide an aesthetic and recreational essential function; thus an urban phytoremediation field trial would be a benefit to the landscape.

Among phytoremediation techniques, phytostabilization and phytoextraction are the most used for PTE. Phytostabilization uses plants and microorganisms to diminish the PTE mobility in soil and transfer them to other compartments, through mechanical and biochemical processes [9]. Within an urban framework, phytostabilization techniques could be used as an initial step towards remediation because, although PTEs could be stabilized, the concentrations do not decrease and, if soil particles are moved (e.g., after wind or water erosion) or change oxidation state (e.g., due to flooding episodes), contaminated particles could exert their toxicity in their new environment. Phytoextraction has, conversely, the aim to remove the contaminants from the soil by either using hyper-accumulator plants [12] or plants with a lower specific uptake of pollutants but high growth rate and biomass production [9,13].

The possible drawback of phytoextraction methods is that usually it requires long reclamation times to decrease total metal concentrations [14]. Based on field biomass production on moderately contaminated soils, phytoextraction would take decades to diminish concentrations to legislative values [9], thus the decrease of the bioavailable fraction would be a more realistic objective [15], as the readily soluble forms of PTE are the most dangerous for the environment [16]. This technique is currently the subject of many laboratory-scale and greenhouse experiments but is still rarely used in field trials, in particular in urban contexts [15,17]. In open field conditions, the success of the phytoextraction depends on time, climate, and on the characteristics of the contaminated matrix.

In a former industrial area, currently embedded into a residential neighborhood, aesthetic appeal is necessary to accept remediation, but the area should be returned to public utility (i.e., as urban park in this case) after restoration and PTE concentration decrease. Consequently, phytostabilization was not feasible in this case, and the phytoextraction approach was chosen.

As hyperaccumulator plants have a very low economic value, for the feasibility of the phytoextraction the use of short rotation coppice crops (SRC) have been promoted in recent years, combining phytoremediation with the production of valuable biomass on the contaminated land [18]. In particular, fast-growing plants from *Populus* and *Salix* genera are the most studied as woody crops and phytoextractors, have been shown to be highly productive [19], mostly in Nordic countries in open-field zones with water supply [20,21].

On the contrary, in the Mediterranean area, with limited water supply and a longer growing season, their performance have been rarely assessed and, to our knowledge, never assessed as a simple tool to remediated contaminated areas within a residential area.

Moreover, beside the remediation process, the SRC method was applied in order to have some of the additional benefits of a green infrastructure: the limitation of access to the contaminated area to the population and the improvement of the aesthetic attractiveness of the area, that is, in this case, undergoing massive restructuration towards the final realization of a public park.

This field experiment was aimed at verifying the usefulness of phytoremediation in a former industrial area located in an urban residential context in terms of heavy metal reclamation, and to verify the possible agronomic management of the parcels in terms of plant selection, watering, and coppicing turnover.

2. Materials and Methods

2.1. Study Area and Plant Selection

The experimentation was conducted in the city of Turin (45°03′ N, 7°40′ E), one of the main urban centers of northern Italy. The Turin climate is humid subtropical (Cfa, according to Köppen–Geiger classification), with cold and wet winters and hot and humid summers. The city is located at 250 m a.s.l., near the Alps, along the river Po. Mean annual precipitation is about 800 mm/year, concentrated mainly in spring and autumn.

The city was characterized by the heavy metallurgical industry and has suffered from de-industrialization over the past three decades. As a consequence, vast areas of former industrial plants were abandoned, and a new urban planning action had to be devised. A large part of the ex- industrial areas was allocated to green infrastructures such as parks and other types of public spaces. However, the restoration of the green areas was confronted with the problem of soil contamination. In the most polluted sites, classical remediation methods were applied, leaving the moderately contaminated sites originating from diffuse pollution. Diffuse pollution appears to be typical of urban areas due to the variety and continuity of sources emitting pollutants. One example is Pb from gasoline [22].

The area selected for the experiment was characterized by heavy metal concentrations (Co, Cr, Cu, Ni, Pb, Zn) above the limits defined for green and residential areas by Italian legislation [23]. This was probably caused by past industrial activities and widespread sources such as traffic for Cu, Ni, Pb, and Zn, while for Co, Cr, and Ni (in part) because of the alluvial deposits upon which the soils developed, as described elsewhere [24,25].

The surface of the experimental plot was about 0.1 ha. The area presented soil excavated in adjacent areas and mixed with around 10% in weight of compost incorporated in the first 15 cm of soil to improve plant rooting and survival ability.

The selected species were chosen between species well adapted to the Piedmont area, having a rapid growth, resulting in large biomass production and tolerant to rather high metal concentrations; species were from *Populus*, *Salix.* and *Robinia* genera.

As comparative studies demonstrated that different clones respond differently to contamination under different pedo-climatic conditions [20,26], three clones of each species were chosen (listed in Table 1). Clones were selected basing on previous studies in the area for their productivity in agricultural soils [27–29] and on previous studies in contaminated soils in Northern Italy for their productivity and tolerance to PTE concentrations (data not published).

Table 1. Species and genotypes used in the experiments.

Code	Genotype	Species
P1	ORION *	*Populus ×canadensis* Mönch
P2	BALDO *	*P. deltoides* Marsh.
P3	VILLAFRANCA *	*P. alba* L.
S1	SI64-017	*Salix alba* L.
S2	MOS310	*S. viminalis* L. (provenance Czech Republic)
S3	DRAGO	*S. babylonica L. x?* (natural hybrid selected by CREA)
R1	NORD	*Robinia pseudoacacia* L. (provenance Northern Italy)
R2	SUD	*R. pseudoacacia* L. (provenance Calabria, Southern Italy)
R3	ENERGY	*R. pseudoacacia* L. (provenance Hungary)

* Clones listed in the Italian Register of Forestry Clones.

Cuttings of *Populus* and *Salix* and 1-year old seedlings of different provenances of *Robinia pseudoacacia* were used.

2.2. Site Preparation and Planting

The selected area was mechanically tilled to a depth of 30 cm and levelled.

Trees were planted following the model of Short Rotation Coppice (SRC) with high density and annual or biennial coppicing. Spacing between trees was 2 × 0.50 m, corresponding to a density of 10,000 trees per hectare (p ha^{-1}). The experimental design was composed of eight randomized blocks and a control area (Figure 1). Each experimental block of 108 m^2 was divided into nine subplots. The allocation of coppicing treatments was randomized and it was annual in the blocks 1-2-5-6, while biennial in the blocks 3-4-7-8.

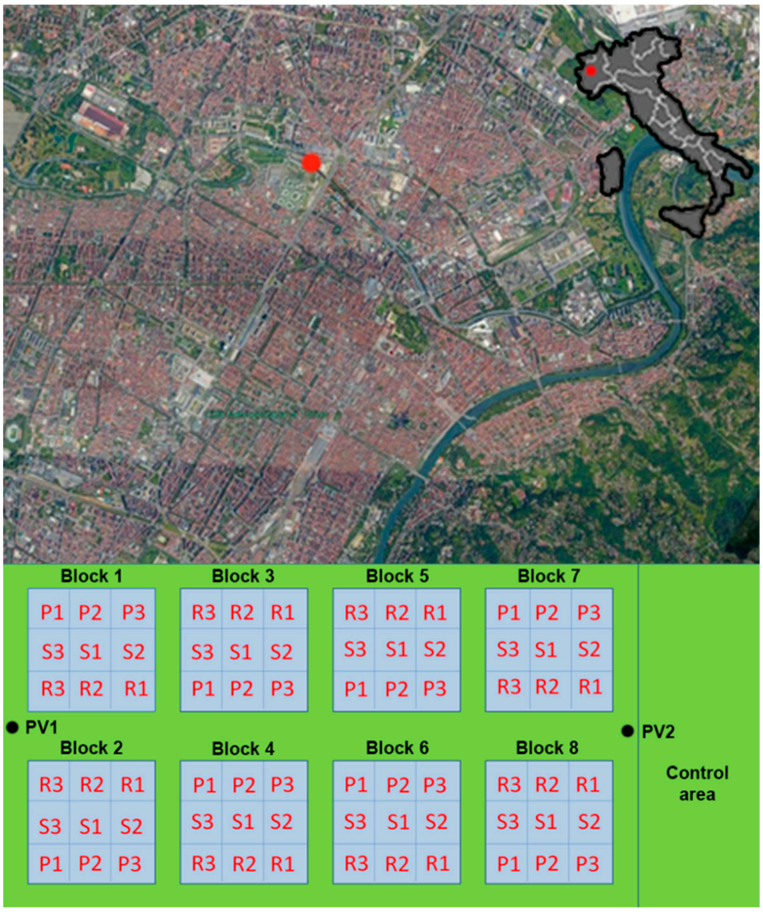

Figure 1. Map of Turin field experiment scheme with randomized blocks, control area, and piezometers positions (PV1, PV2).

In order to evaluate the portion of the metal leached along the soils profile, four lysimeters were installed in the experimental area and one in the control area. To monitor the quality of the groundwater two piezometers, one on the left and one on the right of the experimental area, were installed. The groundwater was analyzed before the study, presenting high values for Ni, due to the rock parent material, and for Cr^{VI}, due to the previous industrial activities. During all the study no noticeable changes in groundwater quality were noted (data reported in Supplementary Materials).

During the first vegetative season, the presence of *Chrysomela populi*, a poplar defoliator insect, was noted at levels that did not require a specific insecticide treatment. Mechanical weed control was carried out, followed by manual hoeing between trees. During summer months three irrigations were carried out.

After the first year, half of the plants (blocks 1-2-5-6) were coppiced. During the second vegetative season, the stumps re-sprouted and the shoots were allowed to grow freely; the development of the foliage and of the roots allowed a better water supply and a greater natural control of the weeds. After the second year trees of all blocks were coppiced.

2.3. Plant Biomass Assessment and Analyses

Tree growth and biomass production are indicative of their adaptation to environmental conditions, both natural and anthropogenic-derived (such as soil pollution).

At the end of the first vegetative season, dendrometric surveys were carried out: total height (h) and diameters at 10 cm from the ground (D_{10}).

During the first harvest the aboveground biomass was freshly weighed in field, and a sample of biomass, equal to one tree per genotype and per block, was dried at 60 °C to constant weight for the determination of the dry matter. Then, the allometric equation: $DW = a \cdot D_{10}^{b}$ was calculated for each genotype considering diameter (D_{10}) and dry weight (DW) of stem and branches, to estimate the biomass of the all plots and of the non-harvested blocks [27,30]. The coefficients are reported in the Supplementary Material (SM), Table S1. From stem and branches, leaves and roots weight were estimated according to previous studies in the same area for *Salix* and *Populus* [28] and for *Robinia* clones [29].

At the end of the second vegetative season a sample of 10 plants per genotype (30 per species), representing the different diametric classes detected, was collected. Tissues were divided on site into leaves, branches, stem, and roots and stored in plastic bags to avoid contamination. Root sampling was limited to coarse roots and the roots were carefully cleaned from soil in the laboratory.

During the summer of both years, two whole plants for each genotype (randomly chosen) were collected for chemical analyses on aboveground (stem, branches, and leaves) and belowground (root system) biomass. All the fresh biomass was washed in abundant 0.01 M EDTA solution to remove material deposited and not absorbed. Samples were dried at 40 °C and ground with a mill. Microwave acid extraction (HNO_3/H_2O_2 4:1 *v*/*v*) was performed on 1.0 g of biomass (Milestone Ethos D, ISO 11466). Pseudo-total content of Co, Cd, Cr, Cu, Ni, Pb, and Zn were determined in all samples by flame atomic absorption spectrometry (FAAS) (Perkin-Elmer AA-400). Accuracy was checked using Standard Reference Materials for plant (NIST SRM 1572, tomato leaves, National Institute of Standards and Technology, Gaithersburg, MD, USA), all recoveries of analyzed metals were between 90% and 110%.

The Bio-Concentration Factor (BCF) was calculated to evaluate the ability of plants to accumulate PTE [31]. BCF is obtained by calculating the ratio between the metal concentration in the plant tissues and the concentration in dry soil (e.g., $BCF_{leaves} = C_{leaves}/C_{soil}$).

Data interpretation and statistical analysis (ANOVA) was carried out using R 3.5.0 program (R Core Team, 2018) and the package "Agricolae: Statistical Procedures for Agricultural Research".

2.4. Soil Sampling and Analyses

Soil was sampled and characterized immediately prior to the trial. In each block, five subsamples were taken using a soil core sampler (one in the center and four in the middle of each half-diagonal) at 0–20 cm depth. The five soil cores were then thoroughly mixed after removing the grass to obtain a homogeneous sample. All samples were dried in the laboratory at 40 °C and passed through a plastic 2-mm sieve prior to laboratory analyses.

At the end of each year, the soil was sampled in correspondence of the plants removed for chemical analyses, to limit the effect of spatial variability in assessing the soil changes due to remediation.

Chemical and agronomic parameters were measured following the procedures officially adopted by the Italian Ministry of Agriculture [32]. All samples were analyzed for pH (1:2.5, soil:water), total carbon (TC) and nitrogen (TN) (CE Instruments, NA2100 Elemental Analyzer), Cation Exchange Capacity (CEC), and carbonates (volumetric method). The particle-size distribution (PSD) was measured with the sieve-pipette method [32].

The bio-accessible metal pool was estimated by extraction with diethylenetriaminepenta-acetic acid (DTPA) [32]. All samples were analyzed for pseudo-total metal content using aqua regia extraction (HCl/HNO3, 3:1 *v*/*v*), performed with microwave digestion of 1.0 g of sample (Milestone Ethos D). The extractable (pseudo-total) content of Cd, Co, Cr, Cu, Ni, Pb and Zn was determined by flame atomic absorption spectrometry (FAAS) (Perkin- Elmer AA-400). Accuracy was verified using Certified Reference Materials for aqua regia-soluble contents in soils (CRM 141R, calcareous loam soil, Community Bureau of Reference, Geel, Belgium). All reagents were of an ultrapure or analytical grade.

2.5. Water Sampling

After major rain events, samples of water were taken from the lysimeters, the water samples were analyzed for pH and metals as above (total Cr, Mn, Cd, Co, Cu, Ni, Pb, and Zn).

During the experimentation, water was taken from the piezometers three times and sent to a certified laboratory (CHELAB di Resana -TV, CSA of Rimini - RN) for the analysis of pH, metals (As, Cr^{VI}, Cr tot, Mn, Cd, Cu, Ni, Pb, and Zn) and total hydrocarbons.

3. Results and Discussion

3.1. Soil Characteristics

The analysis performed at the beginning of the study revealed very homogeneous chemico-physical properties at the experimental site, with all the blocks showing a moderately alkaline pH and a silty sand texture, with 40% skeleton. In Table 2 the average values with standard deviations are reported.

Table 2. Chemico-physical characterization of the soil (n = 32). Average values for the 8 blocks with Standard Deviation (S.D.). Total and diethylenetriaminepenta-acetic acid (DTPA)-extractable (bioavailable) concentrations of metals (in mg/kg) reported along with the Italian legislative limit for green areas.

	pH	**TN**	**TC**	**Org. C**	**$CaCO_3$**	**Sand**	**Silt**	**Clay**	**Skeleton**	**Olsen P**	**CEC**	
		%	%	%	g/kg	%	%	%	%	mg/kg	cmol/kg	
Mean	8.4	0.11	3.4	1.93	123.5	65.3	29.1	5.6	38.2	13.1	5.2	
S.D.	0.1	0.01	0.14		3.5	3	2.9	1	4.4	1.8	0.4	
	Co		**Cr**		**Cu**		**Ni**		**Pb**		**Zn**	
(mg/kg)	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
Mean	28	<0.12	191	<0.5	97	8.3	185	1.3	215	27.7	447	12.6
S.D.	0.6		14		35	1.5	4	0.2	34	2.5	82	12.2
Limit	20		150		120		120		100		150	

TN: Total Nitrogen; TC: Total Carbon; Org. C: Organic Carbon; Olsen P: bioavailable Phosphorus; CEC: Cation Exchange Capacity.

The soil had moderate organic matter content, due to compost addition, and low carbonates. The available P (Olsen P) was low, as were the total nitrogen content and the CEC. The limited fertility of the soil was probably due to the fact that it was excavated from deep layers in the adjacent areas, as a result of urban redevelopment plans.

The pseudo-total concentrations of Co, Cr, Ni, Pb, and Zn in the soil exceeded the legislative limit in all the blocks. However, for Co, Cr, and Ni, the bioavailable concentrations were very low or below the detection limit. This has been often observed in the soils of Turin [33] as the metals are considered to be incorporated in the crystalline lattices of the soil minerals and can scarcely be absorbed by plants. This was in line with the local background values calculated according to the ISO 19258/2005 norm by ARPA Piemonte [34], arising from the parent rock material and from the diffuse historical contamination of the urban area.

Conversely, the concentrations of Pb and Zn suggested an anthropic contribution, with Zn posing the major threats due to its higher concentration. The contamination was probably of historic nature, as the bioavailable fraction of both metals was low [35,36].

3.2. Biomass Production

All the genus tested showed good adaptation to the particular environment. Rooting of trees was complete for all the genotypes employed. Survival rate after the first three months was very high (97% on average), ranging from the 94% of R3, to the 100% of P1 and S2. The differences between genotypes do not reach statistical significance (ANOVA test, $p = 0.05$). Complete data are reported in SM, Tables S2 and S3.

Total biomass productions calculated for one hectare (10,000 plants) for both annual and biennial coppicing methods are reported in Table 3. The equations utilized for aboveground biomass estimation, calculated as reported in the Methods section, are reported in Supplementary Materials (SM).

Table 3. Biomass production at the end of the 1st and 2nd year for each genotype. Average values for blocks (standard deviation) and for species, as dry biomass (in kg per hectare). Different bold letters represent statistical differences ($p = 0.05$).

Genus	Genotype	Annual		Biennial
		1 year	2 year	2 year
Poplar	P1	340 (59)	3335 (29)	2587 (174)
	P2	210 (111)	2447 (42)	1643 (28)
	P3	204 (15)	1228 (50)	1606 (96)
Salix	S1	262 (19)	1360 (80)	2641 (210)
	S2	178 (24)	684 (11)	1440 (135)
	S3	313 (53)	1178 (23)	2671 (112)
Robinia	R1	754 (164)	2684 (37)	4922 (507)
	R2	437 (3)	2274 (67)	3290 (183)
	R3	456 (58)	3597 (144)	4153 (368)
Average				
	Poplar	2592 ± 1132 **a**		1945 ± 556 **a**
	Salix	1321 ± 409 **a**		2251 ± 702 **a**
	Robinia	3395 ± 672 **b**		4122 ± 816 **b**

At the end of the first year, the best productions were achieved by *Robinia* genotypes in both tests, statistically higher than other species, particularly by R1 with 0.75 t ha^{-1} of dry matter (Table 3). *Robinia* was able to withstand a limited water stress, maybe thanks to its symbiosis with nitrogen- fixing bacteria, as shown also from previous studies in the same climate, although on a non- polluted area [29]. *Poplar* and *Salix* gave yields not statistically different from each other (at $p = 0.05$), probably due to water scarcity in the field, as from previous studies in northern Italy with fertilized and irrigated soil, poplars are usually more productive [29,37]. The results were, however, in line with productions made on marginal areas and on contaminated soils [13,17]. In this case, to maximally limit the management of the parcels it was chosen not to irrigate the field unless in the case of extreme drought. Three irrigations were performed during the first year and none during the second.

At the end of the second year both the blocks with annual and biennial coppicing were harvested. During the whole experiment no symptoms of suffering due to excess of pollutants were detected.

The most productive genus was the *Robinia*, with an average production around 4 t ha^{-1}, significantly higher than other species. High yield differences were obtained in all species between genotypes, reflecting the importance in the choice of species suitable to remediate mixed pollution sites but also of the need of a cultivar adapted to the climatic and contamination settings [38].

Genus respond in a different way to coppicing (Table 3); *Poplar* clones were the only ones producing higher biomass during the second year after being coppiced at the end of the first vegetative season.

Salix and *Robinia* produced higher biomass in blocks not coppiced. Due to the high variability between clone production differences between coppicing managements were not statistically significant.

The most productive genotype was the R1 considering biennial coppicing, and R3 in annual coppicing.

Generally, productivity was not higher as in the literature [13,29], probably due to low soil fertility or the limited quantity of water. However, as the plant did not present symptoms of suffering, to increase biomass production a higher planting density is attainable, in order to store higher amounts of PTE in plant tissues. Higher densities are presented in the literature, up to 30,000 plants per hectare [13,17], but with the potential drawbacks of the increase in the operational costs.

3.3. PTE Concentrations in Plant Tissues

During the field experiment, the metal uptake from species was evaluated each year in plants coppiced every year or biennially, to find which method would better balance metal uptake and biomass production.

In Figure 2 the results for Cu and Zn are reported as the most representative contaminants, although Cu concentrations were under the legislative limits. Nickel and Pb were detected in roots and in leaves of some clones, while in the stems their concentration was not detectable (data reported in SM, Table S4). Cadmium and Co values in plants were below the detection limit in most of the samples, in line with the low bioavailable concentrations in soils.

Concentrations of metals in the different tissues are in line with previous studies in moderately contaminated soils [17,39] where it is reported that specific plants concentrate PTE in different parts.

Copper concentrations in leaves rose moderately during the second year in *Poplar* clones and in S2, with higher results in the leaves of the biennial rotation plants. This higher translocation was reflected in the stem of both species, where most of the genotypes have metal concentrations significantly higher in biennial coppiced plants than in annual. This is probably due to the major efficiency of the uptake from roots in undisturbed plants, partially confirmed from the metal concentrations in roots, that are significantly higher in both samples taken at the end of the second year than after the first year.

With regard to Zn, the accumulated concentrations during the second year were significantly higher than during the first year for all clones of *Salix* and *Poplar*. In the leaves, the concentrations in coppiced blocks of the second year were significantly higher than in non coppiced ones. Clones P1, P3, and S2 presented the highest concentrations in leaves, while in stem and roots S2 and S3 genotypes accumulated the highest levels of Zn. In these last tissues, annual and biennial coppicing did not determine statistically different concentrations.

From a management perspective, as stem is the tissue mostly removed in phytoremediation applications (as only few plantations remove leaves from the field), *Salix* clones with biennial coppicing would be the most suitables in terms of relative translocation.

On the contrary, *Robinia* was not an accumulator of Zn, as tissue concentrations were 10 fold lower than *Poplar* and *Salix* clones and did not increase in the second year.

Observed concentrations for *Poplar* and *Salix* species were similar to published studies in mildly contaminated soils [13,40], with a clear increase in PTE uptake during the second year after transplant.

A suitable method to evaluate the ability of plants to accumulate PTE in tissues is the calculation of the bio-concentration factor (BCF) [31]. The only tissue where a concentration (with respect to soil) occurred was leaves, were BCF for both annual and biennial coppicing blocks during the second year were higher than in the first. In order to have the potential to be used as a phitoextractor, a plant should have leaves and stem concentrations of the contaminant higher than the concentration in the soil. In this field trial, concentrations in stem and roots were similar to the concentration of Zn in the soil, thus with a BCF around or lower than 1, probably because of the short duration of the experiment.

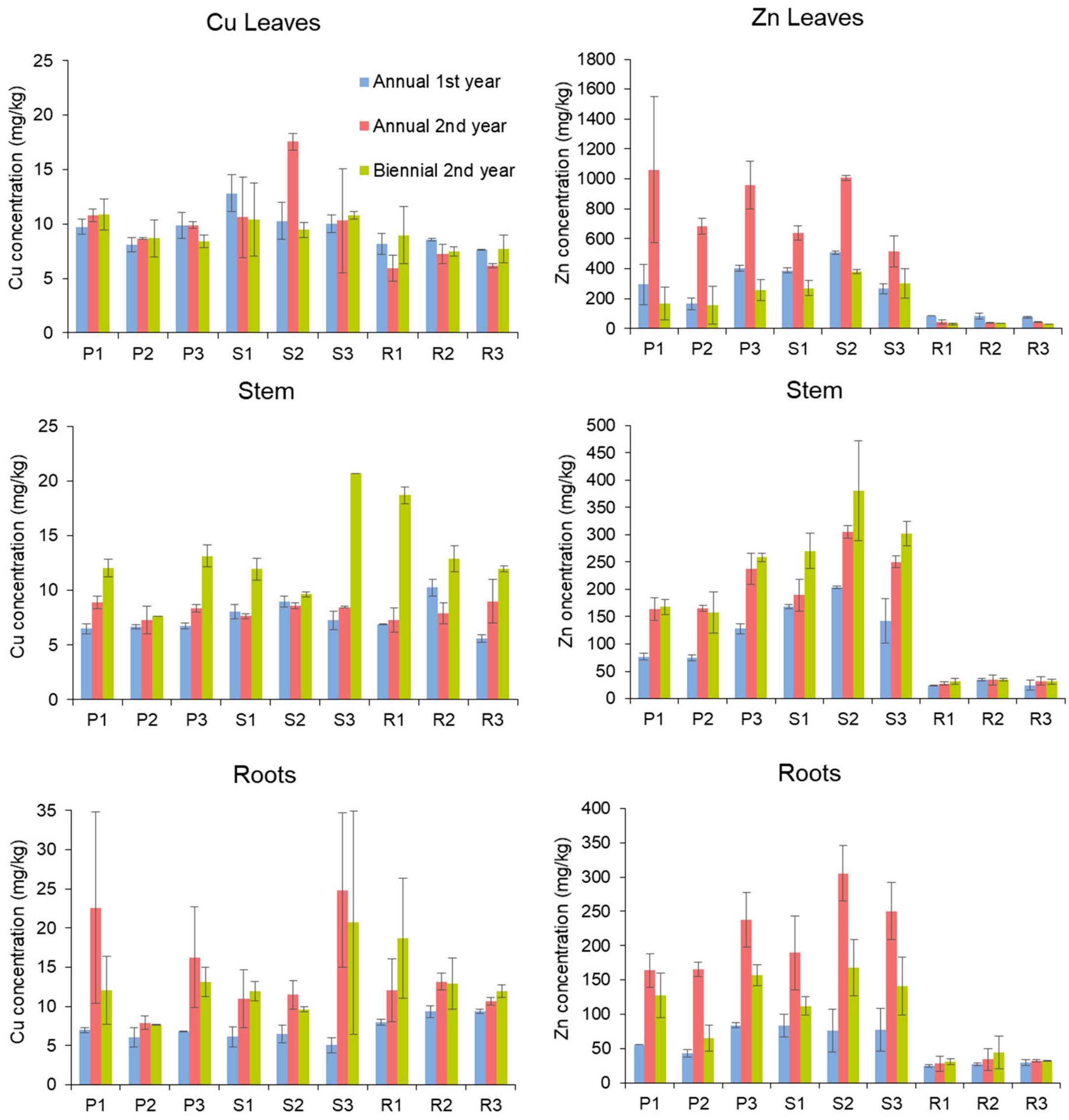

Figure 2. Average Cu and Zn concentrations in the different tissues of the studied species.

3.4. Phytoextraction Potential

The performance of a phytoextraction arises from the combination of biomass production and efficacy of elemental uptake. The phytoextraction rate was calculated for Cu and Zn, the only metals signifacantly uptaken by plants (Figure 3). The sum of the the biomass produced from all the clones during the first year after the transplant was very low, leading to phytoextraction rates considerable lower than during the second year.

Aboveground biomass accumulated in the majority of PTE in all the tested genotypes.

Copper distribution in aboveground biomass was similar for all species. Stem incorporated the majority of PTE in all genotypes (Figure 3), with *Robinia* the most phytoextractor species. *Salix clones* accumulated a higher amount of Cu in stem using biennial coppicing, in the same way as *Robinia*, while in the leaves the uptaken quantities were similar.

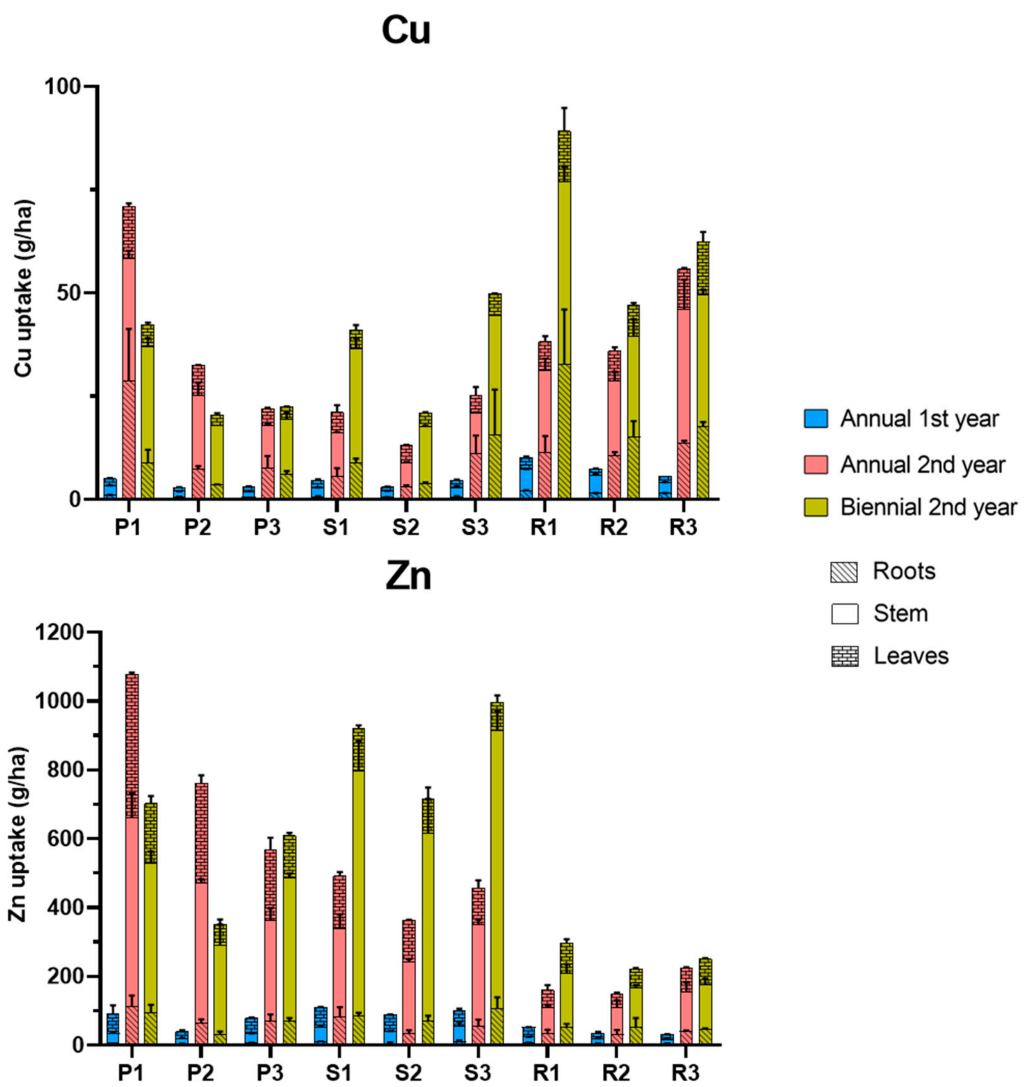

Figure 3. Estimated phytoextraction rates for the studied clones in the three plant tissues. For each clone, tissue uptakes are stacked.

Regarding Zn, during the first year the majority of PTE was present in leaves, while during the second year in stem. Biennial coppiced parcels of *Salix* stored higher amounts of PTE in stem due both to the higher biomass produced and to the higher concentrations. Contrarily, *Poplar* clones (in particular P1) presented similar phytoextraction rates regarding stem values and a significantly higher rate of extraction considering leaves.

Belowground root accumulation of PTE is for interest shown in a phytostabilization experiment, to prevent the leaching of the contaminant to the aquifer or the runoff of it. In this experiment, Zn root uptake was constant over the two years for *Poplar* and *Salix* clones, between 6% and 17% of the total uptake of the plant (Table 4). *Robinia* genotypes, instead, stored higher amounts of metals in roots using biennial coppicing (up to the 23% of the total), mostly due to the higher root biomass developed under this management.

Table 4. Total and bioavailable potentially toxic element (PTE) concentrations in soil before and after the field trial. Standard deviation in parenthesis, n = 4 for each block. In bold statistically different concentrations before and after the trial.

		Total concentrations (mg/kg)						
		Cd	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
P	Before	1.7 (0.08)	27.9 (1.1)	195 (15)	96 (32)	185 (3)	203 (25)	421 (80)
	After	0.4 (0.16)	27.3 (0.5)	229 (19)	88 (8)	198 (16)	2017 (70)	552 (123)
S	Before	1.6 (0.12)	27.7 (0.4)	191 (16)	92 (31)	186 (4)	227 (47)	396 (70)
	After	0.2 (0.17)	27.4 (1.0)	213 (208)	107 (17)	213 (29)	208 (51)	490 (95)
R	Before	1.6 (0.13)	28.1 (0.6)	206 (22)	81 (9)	186 (5)	193 (32)	442 (133)
	After	0.2 (0.26)	27.3 (0.9)	223 (34)	109 (37)	209 (20)	205 (68)	456 (63)
Control		1.8	31.8	76	165	216	146	413
		DTPA-Extractable concentrations (mg/kg)						
		Cd	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
P	Before	<0.09	<0.12	<0.5	9.5 (1.0)	1.6 (0.17)	23.3 (2.3)	25.5 (1.9)
	After	<0.09	<0.12	<0.5	8.3 (2.3)	0.7 (0.25)	31.6 (13.5)	18.4 (6.5)
S	Before	<0.09	<0.12	<0.5	10.1 (0.8)	1.6 (0.07)	26.4 (2.7)	**26.2 (2.9)**
	After	<0.09	<0.12	<0.5	7.8 (2.2)	0.9 (0.42)	34.1 (8.1)	**16.7 (2.4)**
R	Before	<0.09	<0.12	<0.5	9.7 (1.0)	1.6 (0.20)	23.7 (2.8)	**27.8 (4.4)**
	After	<0.09	<0.12	<0.5	7.7 (2.2)	1.5 (0.63)	31.5 (9.9)	**20.7 (4.2)**
Control		0.08 (0.03)	-	-	6.5 (0.4)	1.15 (0.53)	26.4 (3.7)	29 (17.7)

Root accumulation of Cu was more prominent than for Zn, in particular for annually coppiced plants during the second year, with up to the 44% of the Cu allocated in root biomass.

Pooling all these data, we calculated the total uptake from plants, reported in Figure 3 and in Table S5 as average values for species. *Robinia* clones were the most productive species for Cu phytoextraction (Figure 3), particularly with biennial coppicing, due to their greater biomass production.

With this management, the more productive clone for Cu phytoextraction was R1, while using annual coppicing P1 yielded better results, although slightly lower than biennial coppiced R1.

Zinc phytoextraction was more important, bearing the results in line with literature studies during field trials [13,17]. Accumulation yields were higher with annual coppicing for poplar clones, with P1 having the highest phytoextraction rate during the second year (1077 g ha^{-1}). *Salix* and *Robinia* clones performed better on biennial management, with clones S1 and S3 extracting, respectively, 919 and 997 g ha^{-1} of zinc, not statistically different from P1.

This total uptake did account also for the root system, containing between 9% and 11% of the Zn and not removed in phytoremediation trials. However, for the calculation of the translocation of PTEs in plant tissues it is of primary importance in the management of a trial and is evident from Figure 3 that leaves carry an important share of the PTE uptake. In particular, although the total uptake was not so large, the allocation in leaves represented 39% of the accumulation in clone P1, while in the case of willows 13% and the 8%, respectively, in S1 and S3 clone.

In this case, therefore, a successful phytoremediation should include the collection of leaves, a practice little used in field trials, as it will increase costs. In an urban context the collection will be easier, as the city is already acquainted with the collection of leaves on the streets and is thus equipped.

3.5. Soil PTE Concentrations

The total and the bioavailable concentrations of PTE in the studied soil before and after the field trial are reported in Table 4.

Although two years is a limited time for a phytoremediation process, the bioavailable concentrations of the two metals preferentially uptaken by plants (Cu and Zn) decreased for all species. The decrease for Zn was statistically significant for all clones at the end of the experiment and, on average, *Poplar* clones reduced Zn by 28%, *Salix* by 36%, and *Robinia* 26%.

Contrarily, available Pb concentrations seem to increase during the trial (although not statistically significant).

The total PTE concentrations did not change during the trial, consistent with literature results, where a decrease has been rarely described in short term experiments [17].

Possible release of PTE in soil-porewater and groundwater was monitored. The soil solution was basic, with an average pH of 8.0; PTE concentrations were below the detection limits of the instruments during the whole of the trial.

In the groundwater, Cr^{VI} and Ni contents were higher than the limits defined by Italian law for green areas before and during the experiment (reported in Supplementary Materials, Table S6). These high concentrations could probably be attributed to the past industrial use of this area, leading to a relict contamination. However, considering the analysis on interstitial water and groundwater, a leaching process induced by the phytoremediation can be excluded.

4. Conclusions

Short rotation coppice phytoremediation was shown to be a promising method in an urban context to improve the landscape and to limit access to the contaminated area. All the tested plant genotypes showed good adaptation to the particular environment. *Robinia* clones were demonstrated to be the most productive species in field conditions, probably due to the poor fertility of the soil, with respect to an agricultural soil, and to drought.

Coppicing management modified the biomass relationships between roots, stem, and leaves. This, together with the differences in accumulation and translocation behavior of the genotypes, made evident the need, prior to a field trial, of a study to identify the management approach and suitable genotypes. Leaves contained a large part of the extracted PTE, thus a successful phytoremediation should include the collection of leaves, for example through the use of harvesting nets. *Poplar* and *Salix* species proved to be suitable for the field conditions (in particular clones P1, S1, and S3), diminished significantly the bioavailable concentrations of Zn in the soil. Conversely, in field conditions the 2-years of the project were not sufficient to diminish the total PTE concentration in the soil. Improving the biomass yield through phytomanagement options (fertilization, irrigation, coppicing, etc.) could make SRC phytoremediation an economic and effective solution to manage urban contaminated areas, coupling the added values of biomass production to the landscape benefits.

Supplementary Materials: The Supplementary Materials are available online at http://www.mdpi.com/2076-3417/10/1/307/s1, Table S1 Table S1. Coefficient of the equation to estimate aboveground biomass per tree (DW) in g from diameter at 10 cm of height (D10) in the first and second year of growth, Table S2: Table S2. Survival percentage (Sur), number of sprouts per stump (ns/s) and stem dry biomass yield at the end of first year in Mg per hectare, per genotypes and turnover coppicing, Table S3: Table S3. Survival percentage (Sur), number of sprouts per stump (ns/s) and stem dry biomass yield at the end of second year (B1+2) in Mg per hectare, per genotypes and turnover coppicing, Table S4: Table S4. Mean total trace element concentrations in leaves, stem, and roots of the nine cultivars for both harvesting seasons, Table S5: Table S5. Average Zn and Cu phytoextracted (calculated as g ha^{-1}) and distribution among aboveground and belowground tissues, Table S6: Table S6. Ground water analysis, piezometers PV1 and PV2; Figure S1. Photo of the area before the remediation process and at the end of the first vegetative season.

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References

1. Ajmone Marsan, F.; Biasioli, M. Heavy metals in urban soils. *Water Air Soil Pollut.* **2010**, *213*, 121–143.
2. Luo, X.S.; Yu, S.; Zhu, Y.G.; Li, X.D. Trace metal contamination in urban soils of China. *Sci. Total Environ.* **2012**, *421*, 17–30. [CrossRef] [PubMed]
3. Pan, L.; Wang, Y.; Ma, J.; Hu, Y.; Su, B.; Fang, G.; Wang, L. A review of heavy metal pollution levels and health risk assessment of urban soils in Chinese cities. *Environ. Sci. Pollut. Res.* **2018**, *25*, 1055–1069. [CrossRef] [PubMed]
4. Meuser, H. Environmental Pollution 18. In *Contaminated Urban Soils*; Springer: Dordrecht, The Netherlands, 2010.
5. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals-concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [CrossRef] [PubMed]
6. EEA, Progress in Management of Contaminated Sites. Available online: https://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment/view (accessed on 8 May 2019).
7. Batty, L.C.; Dolan, C. The potential use of phytoremediation for sites with mixed organic and inorganic contamination. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43*, 217–259. [CrossRef]
8. Rulkens, W.H.; Tichy, R.; Grotenhuis, J.T.C. Remediation of polluted soil and sediment: Perspectives and failures. *Water Sci. Technol.* **1998**, *37*, 27–35. [CrossRef]
9. Mench, M.; Lepp, N.; Bert, V.; Schwitzguébel, J.P.; Gawronski, S.W.; Schröder, P.; Vangronsveld, J. Successes and limitations of phytotechnologies at field scale: Outcomes, assessment and outlook from COST Action 859. *J. Soils Sediments* **2010**, *10*, 1039–1070. [CrossRef]
10. Biasioli, M.; Ajmone-Marsan, F. Organic and inorganic diffuse contamination in urban soils: The case of Torino (Italy). *J. Environ. Monit.* **2007**, *9*, 862–868. [CrossRef]
11. Jacobs, A.; De Brabandere, L.; Drouet, T.; Sterckeman, T.; Noret, N. Phytoextraction of Cd and Zn with Noccaea caerulescens for urban soil remediation: Influence of nitrogen fertilization and planting density. *Ecol. Eng.* **2018**, *116*, 178–187. [CrossRef]
12. Dickinson, N.M.; Baker, A.J.M.; Doronila, A.; Laidlaw, S.; Reeves, R.D. Phytoremediation of inorganics: Realism and synergies. *Int. J. Phytoremediation* **2009**, *11*, 97–114. [CrossRef]
13. Courchesne, F.; Turmel, M.C.; Cloutier-Hurteau, B.; Constantineau, S.; Munro, L.; Labrecque, M. Phytoextraction of soil trace elements by willow during a phytoremediation trial in Southern Québec, Canada. *Int. J. Phytoremediation* **2017**, *19*, 545–554. [CrossRef] [PubMed]
14. Morel, J.L.; Echevarria, G.; Goncharova, N. NATO Science Series IV: Earth and Environmental Sciences. In *Phytoremediation of Metal-Contaminated Soils*; Springer: New York, NY, USA, 2006.
15. Robinson, B.H.; Anderson, C.W.N.; Dickinson, N.M. Phytoextraction: where's the action? *J. Geochem. Explor.* **2015**, *151*, 34–40. [CrossRef]
16. Fischerova, Z.; Tlustos, P.; Szakova, J.; Sichorova, K. A comparison of phytoremediation capability of selected plant species for given trace elements. *Environ. Pollut.* **2006**, *144*, 93–100. [CrossRef] [PubMed]
17. Guidi Nissim, W.; Palm, E.; Mancuso, S.; Azzarello, E. Trace element phytoextraction from contaminated soil: A case study under Mediterranean climate. *Environ. Sci. Pollut. Res.* **2018**, *25*, 9114–9131. [CrossRef] [PubMed]
18. Desjardins, D.; Brereton, N.J.B.; Marchand, L.; Brisson, J.; Pitre, F.E.; Labrecque, M. Complementarity of three distinctive phytoremediation crops for multiple-trace element contaminated soil. *Sci. Total Environ.* **2018**, *610*, 1428–1438. [CrossRef]
19. Pajević, S.; Borišev, M.; Nikolić, N.; Arsenov, D.D.; Orlović, S.; Župunski, M. Phytoextraction of Heavy Metals by Fast-Growing Trees: A Review. In *Phytoremediation*; Ansari, A., Gill, S., Gill, R., Lanza, G., Newman, L., Eds.; Springer: Berlin, Germany, 2016.
20. Labrecque, M.; Teodorescu, T.I. Field performance and biomass production of 12 willow and poplar clones in short-rotation coppice in southern Quebec (Canada). *Biomass Bioenergy* **2005**, *29*, 1–9. [CrossRef]
21. Pérez-Cruzado, C.; Sanchez-Ron, D.; Rodríguez-Soalleiro, R.; Hernández, M.J.; Sánchez-Martín, M.; Cañellas, I. Biomass production assessment from Populus spp. short-rotation irrigated crops in Spain. *GCB Bioenergy* **2014**, *6*, 312–326. [CrossRef]
22. Facchetti, S. Lead in petrol. The isotopic lead experiment. *Acc. Chem. Res.* **1989**, *22*, 10–370. [CrossRef]

23. Italian Republic. Decree of the Ministry of the Environment, Land and Sea Protection (MATTM) 03/04/2006 “Norme in material ambientale”. *Official Journal of the Italian Republic*, 14 April 2006; p. 88.
24. Ajmone-Marsan, F.; Biasioli, M.; Kralj, T.; Grcman, H.; Davidson, C.M.; Hursthouse, A.S.; Madrid, L.; Rodrigues, S. Metals in particle-size fractions of the soils of five European cities. *Environ. Pollut.* **2008**, *152*, 73–81. [CrossRef]
25. Bonifacio, E.; Falsone, G.; Piazza, S. Linking Ni and Cr concentrations to soil mineralogy: Does it help to assess metal contamination when the natural background is high? *J. Soils Sediments* **2010**, *10*, 1475–1486. [CrossRef]
26. Wu, F.; Yang, W.; Zhang, J.; Zhou, L. Cadmium accumulation and growth responses of a poplar (Populus deltoides x Populus nigra) in cadmium contaminated purple soil and alluvial soil. *J. Hazard. Mater.* **2010**, *177*, 268–273. [CrossRef] [PubMed]
27. Facciotto, G.; Zenone, T.; Failla, O. Aboveground biomass estimation for Italian poplar SRF. In Proceedings of the 14th European Conference & Exhibition, Biomass for Energy, Industry and Climate Protection, Paris, France, 17–21 October 2005; pp. 298–299.
28. Bergante, S.; Facciotto, G. Yields of poplar SRC and VRC grown with different fertilization and irrigation management. In Proceedings of the 23th European Biomass Conference & Exhibition, Wien, Austria, 1–4 June 2015; pp. 214–218.
29. Mantovani, D.; Veste, M.; Boldt-Burisch, K.; Fritsch, S.; Koning, L.A.; Freese, D. Carbon allocation, nodulation, and biological nitrogen fixation of black locust (*Robinia pseudoacacia L.*) under soil water limitation. *Ann. For. Res.* **2015**, *58*, 259–274. [CrossRef]
30. Verwijst, T. and Telenius, B. Biomass estimation procedures in short rotation forestry. *For. Ecol. Manag.* **1999**, *121*, 137–146. [CrossRef]
31. Yoon, J.; Cao, X.; Zhou, Q.; Ma, L.Q. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* **2006**, *368*, 456–464. [CrossRef]
32. Italian Republic. Decree of the Ministry of Agricultural and Forestry Policies (MiPAF). “Metodi ufficiali di analisi chimica del suolo”. *Official Journal of the Italian Republic, Ordinary Supplement*, 21 October 1999; p. 248.
33. Madrid, F.; Biasioli, M.; Ajmone-Marsan, F. Availability and Bioaccessibility of Metals in Fine Particles of Some Urban Soils. *Arch. Environ. Contam. Toxicol.* **2008**, *55*, 21–32. [CrossRef]
34. Piemonte, A.R.P.A. Relazione Sullo Stato Dell’ambiente in Piemonte. 2018. Available online: http://relazione.ambiente.piemonte.gov.it (accessed on 29 November 2019).
35. Ma, Y.; Lombi, E.; Oliver, I.W.; Nolan, A.L.; McLaughlin, M.J. Long-Term Aging of Copper Added to Soils. *Environ. Sci. Technol.* **2006**, *40*, 6310–6317. [CrossRef]
36. Liang, S.; Guan, D.; Li, J.; Zhou, C.; Luo, J.; Ma, L.Q. Effect of aging on bioaccessibility of arsenic and lead in soils. *Chemosphere* **2016**, *151*, 94–100. [CrossRef]
37. Bergante, S.; Facciotto, G. Impianti annuali, biennali, quinquennali. *SHERWOOD-Foreste ed Alberi Oggi* **2006**, *128*, 25–36.
38. Grenier, V.; Pitre, F.E.; Guidi Nissim, W.; Labrecque, M. Genotypic differences explain most of the response of willow cultivars to petroleum contaminated soil. *Trees Struct. Funct.* **2015**, *29*, 871–881. [CrossRef]
39. Zárubová, P.; Hejcman, M.; Vondráčková, S.; Mrnka, L.; Száková, J.; Tlustoš, P. Distribution of P, K, Ca, Mg, Cd, Cu, Fe, Mn, Pb and Zn in wood and bark age classes of willows and poplars used for phytoextraction on soils contaminated by risk elements. *Environ. Sci. Poll. Res.* **2015**, *22*, 18801–18813. [CrossRef] [PubMed]
40. Wahsha, M.; Bini, C.; Argese, E.; Minello, F.; Fontana, S.; Wahsheh, H. Heavy metals accumulation in willows growing on Spolic Technosols from the abandoned Imperina Valley mine in Italy. *J. Geochem. Explor.* **2012**, *123*, 19–24. [CrossRef]