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Trace Metal Accumulation and Phytoremediation Potential of Four Crop Plants Cultivated on Pure Sewage Sludge

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Abstract: Phytoremediation is a viable strategy to remove trace metal contaminants from sewage sludge but still is poorly investigated. The aim of this study was to quantify the trace metal removal of *B. napus*, *B. juncea*, *H. annuus*, *Z. mays* grown on pure sewage sludge. Each species was grown on six different sewage sludge for 8 weeks and sludge were analysed for trace metal content and physico-chemical characteristics. Our results confirmed that all the tested sludge supported plant growth. The tested sludge showed a plant vigorousness lower (46% of sludge) or similar/increased (54% of sludge) compared to control treatment. *B. juncea* and *B. napus* were the most efficient species in the bioaccumulation, of trace metals. The average percentage of metals removed by the selected species was 0.2% for As, 0.85% for Cd, 0.09% for Cr, 0.36% for Cu, 0.36% for Ni, 4.2% for Se, 1.2% for Zn. In conclusion, our results showed that phytoremediation can be applied to sewage sludge, despite the chosen species have low efficiency in trace element removal. Further studies using hyperaccumulator species are needed which may lead to a higher efficiency of the process opening up new possibilities for the management strategies of this waste.

Keywords: metals; metalloids; phytotechnology; crop plants; wastewater treatment

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1. Introduction

Sewage sludge (SS) is a solid or semi-solid residue of the wastewater treatment process, consisting of sand, organic solids, and bacterial biomass, together with organic and inorganic pollutants [1]. SS is highly enriched in organic matter (up to 50–70% dry weight, dw) and is characterized by significant amounts of N and P (around 3% and 2% dw, respectively), and micronutrients for plant growth (e.g., Cu and Zn) [2]. Nonetheless, it can also contain hazardous contaminants, such as organic pollutants (e.g., polycyclic aromatic hydrocarbons, pesticides, pharmaceuticals) [3], animal pathogens (e.g., *Salmonella* spp., *Escherichia coli*, *Clostridium* spp.), plant pathogens (*Xanthomonas* spp., *Agrobacterium* spp.) and trace metals [4].

Sewage sludge production is rapidly increasing due to urbanization and demographic growth: in Europe, the amount of sludge produced is estimated at around 10 million tons annually, of which 1.1 million tons in Italy alone [5]. Hence, the need for more sustainable SS management has become a global challenge in which reuse and recycling, as well as investigation of strategies to reduce the potentially harmful effects on human health and the environment, are widely encouraged [6], as observed in other research work on phytoremediation field [7].

The most common SS management methods are landfilling, incineration, and land disposal/agricultural use [1]. Landfill disposal is usually discouraged, as it does not allow the recovery of valuable nutrients (e.g., N, P, Mg) [8]. Moreover, this strategy is known to cause substantial CO_2 emission and leachate production, leading to groundwater and soil contamination [6]. Incineration, is widely practiced, due to the increased efficiency of incineration plants and the possibility to produce thermal energy [9]. Land disposal constitutes one of the most accepted and extensively used management strategies. In Europe, around 40% of sludge produced is intended for agriculture [10]. In fact, due to the high concentration of organic matter, N, P and micronutrients, SS use in agriculture recycles these nutrients, thus representing a good alternative to traditional chemical fertilizers. Nevertheless, a main obstacle to this disposal strategy can be the presence of high concentrations of hazardous contaminants in some sludge [11].

To prevent harmful consequences on human health and on the environment, the Sewage Sludge European Directive (Directive 86/278/EEC) [12] regulates SS management since 1986 by setting up the requirements and limits for SS use in agriculture [13]. More stringent regulations are, however, applied in different European countries, such as in Italy (Legislative Decree 99/1992) [14]. The Legislative Decree 99/92 establishes the minimum SS nutrient content and sets the limits for the presence of harmful substances. In particular, total organic carbon (TOC) must be >20% dw, N > 1.5% dw and P > 0.4% dw; conversely, Cd, Ni, Pb, Cu, and Zn are not allowed to exceed 20, 300, 750, 1000 and 2500 mg/kg dw, respectively. Other Italian regulations (Legislative Decree 109/2018) [15] directly affect SS land disposal by updating limits for other pollutants, among which Se (<10 mg/kg dw), As (<20 mg/kg dw), total Cr (<200 mg/kg dw), CrVI (<2 mg/kg dw) and hydrocarbons (<1000 mg/kg dw). Overall, this regulatory framework is aimed at promoting SS reuse, while reducing its possible hazardous effects, according to the principles of a circular economy.

Among all contaminants, metal ions, both essential (e.g., Zn, Cu) and non-essential (e.g., Cd, As, Ni), represent the main limit to SS reuse in agriculture. Their concentrations in sludge are variable due to differences in wastewater origin and treatment. Sörme & Lagerkvist [16] found that Cu, Zn and Cd contributions to wastewater originated from households' tubes and pipes, car washes and roof run offs. Lead (Pb), on the other hand, was hypothesized to derive from street dust being washed away by stormwater. Finally, other specific metals, such as Ni and Cr, could originate from galvanic industries in the area served by the wastewater treatment plant [17].

Since metal contamination is so common and problematic, developing new methods to reduce SS metal concentrations within the limits for agricultural reuse is nowadays a challenging goal. To this extent phytoremediation may represent a viable strategy to clean up SS despite its application to this type of waste has been poorly investigated. The selection of this method was guided by the fact that, in comparison to other strategies used for trace metals' removal (such as chemical leaching), phytoremediation is more economically and environmentally sustainable [18].

The term phytoremediation refers to a remediation strategy taking advantage of some plants' capacity to absorb pollutants, thereby removing them from the environment [19]. Among the different phytoremediation strategies, phytoextraction is the main and most promising approach used to remove metals from contaminated soils [18].

Both natural hyperaccumulators and crop species can be used in phytoextraction. Hyperaccumulators are plants that grow on metal-contaminate soil [20] and have high metal tolerance and bioaccumulation rates but are slow-growing and low biomass-producing species. On the other hand, crop species have low bioaccumulation rates, compensated by the production of large volumes of harvestable biomass, while having minimal growth requirements [21]. Previous research identified *Brassica napus* L., *Brassica juncea* (L.) Czern, *Helianthus annuus* L. and *Zea mays* L. as the most suitable candidates for As, Cd, Ni, Cr, Cu, Se, Mn, Zn, and Pb removal [22–25].

Several studies have evaluated the growth and metal accumulation capacity of these crops when cultivated on sludge-amended soils. Belhaj et al. [26] tested *H. annuus* on soils amended with 2.5, 5, and 7.5% SS and reported an increased biomass production (11, 16, and 29%, respectively) and a conspicuous accumulation of Cr (0.2 mg/kg dw), Ni (0.1 mg/kg dw), Cu (0.08 mg/kg dw) and Zn (60 mg/kg dw). Similar results were reported for *Z. mays* cultivated on soil amended with 30, 75, 150, and 300 t/ha SS, showing an increased biomass production (up to 121% for shoots) and accumulation of Cu (11.5 mg/kg dw), Pb (1.5 mg/kg dw) and Zn (445 mg/kg dw) in stems [27]. *B. juncea* has been identified as a metal tolerant species, with accumulation rates up to 40 mg/kg dw for Pb, 8 mg/kg dw for Cd and 300 mg/kg dw for Zn when cultivated on sludge-amended soils [28].

Previous reports demonstrated that phytoextraction can indeed constitute a viable method to clean up metal-polluted SS before its final agricultural utilization [29]. However, despite the available data on the phytoremediation potential of crop plants grown on SS-amended soils [30–32], little information is currently available regarding the feasibility and efficiency of this practice in removing contaminants from pure SS [29,33]. In fact, even though SS has been proven to be beneficial to plants when used as organic amendment, it can conversely become toxic when used as sole growing medium. Further studies are thus needed to find out whether phytoremediation can also be successfully applied to pure SS.

In this context, the present study aimed at investigating the growth and metal phytoextraction potential of four selected crop species, *Z. mays*, *H. annuus*, *B. napus* and *B. juncea*, cultivated on pure SS coming from six different wastewater treatment plants (WWTPs). These crop-species were selected based on their simple growth requirements and high tolerance to a wide range of soil conditions, making them the most appropriate candidates to grow on sludge in assessing the potential of this new approach. The main novelty of this study resides in the application of phytoremediation for the removal of As, Cd, Ni, Cr, Cu, Se, Mn, Zn, Pb from pure undiluted SS.

2. Materials and Methods

2.1. Sewage Sludge Collection and Preparation

Sewage sludge samples were collected between December 2019 and March 2020 at 6 different wastewater treatment plants (WWTPs) of the Emilia-Romagna region in northern Italy. The six WWTPs, will hereafter be indicated as P1 (N44.5517839, E11.3639399), P2 (N44.6766200, E10.9440777), P3 (N44.4127884, E11.5812478), P4 (N44.5755616, E10.8000404), P5 (N44.8740183, E10.0787511) and P6 (N44.8289575, E10.0155350), to ensure anonymity of the locations as requested by the multiutility management. The plants are heterogeneous in terms of size, wastewater collected and final sludge stabilization process. P1 and P2 are the biggest plants with a population equivalent (PE) of 800 and 500 thousand people, respectively. In these plants, final sludge stabilization is performed by means of continuous anaerobic digestion at 35 °C. P3, P4, P5 and P6 are smaller plants, with 70, 50, 30, and 24 thousand PE, respectively, in which the final treatment consists of aerobic stabilization.

The type of final stabilization largely influences SS characteristics: after anaerobic digestion, SS was found to be dryer and more mineralized compared to aerobically stabilized SS, which had a higher water and organic matter content. Each WWTP was sampled twice, two months apart, to take into consideration sludge variability. The sludge was collected after the dehydration centrifuges, from the container used for sludge storage prior to disposal. Every sample consisted of 50 kg of solid SS, with a water content of around 70–75% (*w*/*w*). Samples were kept in closed plastic bags at room temperature until use. Before use, sludge from the two sub-samples from the same WWTP was carefully mixed to obtain a single homogeneous sample. Each sample was aerated for 24 h, by spreading it in a thin layer on a plastic sheet outside. Once aerated 8 kg of SS from each sample were placed in 40 × 30 × 20 cm undrilled plastic boxes (four replicates for each SS type). Each box was endowed with an inspection pipe (ø 4 cm) placed in a corner, which assured inspection of the box bottom to prevent water stagnation (Supplementary Figure S1).

2.2. Seedling Transfer

The crop plants *Z. mays* L. (var. Kursus), *H. annuus* L. (var. ES mobidic), *B. napus* L. (var. Oleifera) and *B. juncea* L. (var. Brons) were tested as potential candidates for sludge phytoremediation. Seeds were taken from the Department of Agricultural and Food

Sciences, University of Bologna. Before sowing, seeds were hydrated overnight in tap water. Seeds were then planted on pure peat in ø 3.5 cm seedling boxes (1 seed/cell) and kept at 20 ± 1 °C, with a 16/8 h: light/dark photoperiod, until germination.

One week after germination, 11 plants of the same species were transferred to one of the 4 replicate boxes for each SS type to have all species growing on every SS type. Four boxes filled with 50% (*w*/*w*) compost and 50% (*w*/*w*) peat served as negative controls. Seedling transfer was done in March and plants were cultivated for 8 weeks in a glass house under natural light conditions at a temperature of 20 ± 1 °C. Boxes were watered 3 times per week with a variable amount of deionized water using the inspection pipe to detect any water left from previous irrigations.

2.3. Sample Collection and Preparation

Sewage sludge samples were collected before and after plant cultivation. Raw sludge was collected at the end of the aeration and homogenization process, whereas remediated sludge was collected at the end of the plant cultivation period (8 weeks). After 8 weeks of growth, plants were harvested, collecting shoots and roots separately. Root total biomass was not determined, since it was unfeasible to remove the finer roots from the sludge.

Roots and shoots were washed under tap water until complete removal of sludge particles and, then, rinsed with deionized water. To calculate dw, sludge and plant samples were placed in a ventilated oven at 105 °C for 24 h until constant weight. Plant parts were powdered in an A11 basic analytical mill (IKA, Staufen, Germany) to obtain a homogeneous sample. SS samples were instead placed in a MM 400 ball-mill (Retsch GmbH, Haan, Germany) and grinded at 20 shake/s for one minute to obtain a fine powder.

2.4. Total and Bioavailable Metal Quantification

The bioavailable trace metals were quantified in sludge samples by means of diethylenetriaminepentaacetic acid (DTPA) extraction [34]. The extracting solution was composed of 14.92 g/L triethanolamine (TEA), 1.47 g/L $CaCl_2 \cdot 2H_2O$ and 1.97 g/L DTPA, adjusted to pH 7.3 ± 0.5 with 2 M HCl. Amounts of 1.5 g dw of sludge powder were extracted with 50 mL of DTPA solution and placed in a shaker for 2 h. The samples were filtered over Whatman 42 (Maidstone, UK) ashless filter paper before ICP-OES analysis.

Aqua regia digestion was performed for SS total metal quantification following a modified method from EPA-ROC [35]. Sludge powders (0.25 g dw) were placed in TFM digestion tubes for close vessel digestion together with 6 mL of 37% (*v*/*v*) HCl, 2 mL of 69% (*v*/*v*) HNO_3 and 0.5 mL of 35% (*v*/*v*) H_2O_2. All reagents were Suprapur grade purchased from Merck (Darmstadt, Germany). Six tubes at a time were placed into a START D microwave digestion system (Milestone, Sorisole, BG, Italy) and subjected for 40 min to the following digestion cycle: 2 min at 250 Watt, 2 min at 400 Watt, 1 min at 0 Watt, 3 min at 750 Watt, and 32 min cooling.

Analogously, plant sample digestion was performed starting from 0.25 g dw of root or shoot powder placed in TFM digestion tubes with 6 mL of 69% (*v*/*v*) HNO_3 and 0.5 mL 35% (*v*/*v*) H_2O_2 [36]. Six of them at a time were subjected for 40 min to the following microwave digestion cycle: 2 min at 250 Watt, 2 min at 400 Watt, 1 min at 0 Watt, 2 min at 600 Watt, and 33 min cooling. After digestion, samples were brought up to the volume of 20 mL with milliQ water and filtered over Whatman 42 (Maidstone, UK) ashless filter paper. The quantification of trace elements was carried out with a Spectro Arcos ICP-OES (Ametek, Berwyn, PA, USA).

The limits of quantification of the analyzed elements were 0.006753 mg/kg dw (As), 0.000373 mg/kg dw (Cd), 0.00042 mg/kg dw (Cr), 0.000542 mg/kg dw (Cu), 0.000309 mg/kg dw (Ni), 0.001703 mg/kg dw (Pb), 0.011287 mg/kg dw (Se) and 0.000375 mg/kg dw (Zn). For quality control, three replicates of BCR®—143 municipal sludge certified reference material (CRM) (JRC-Joint Research Centre, Geel, Belgium) and five replicates of BCR®—060 aquatic plant (*Lagarosiphon major* (Ridl.) Moss.) CRM (JRC-Joint Research Centre, Geel, Belgium) were digested together with sludge and plant samples, respectively. Recovery rates of all metals were within ±5% of the CRM target concentrations. Quality control solutions were also included in the measurements to assure instrumental measurement stability. Data are expressed as mg of metal per kg of sample dry weight (mg/kg dw).

2.5. pH and Conductivity Analysis

Raw and remediated sludge samples were tested for pH and electrical conductivity (EC). For pH and EC measurement, 3 and 5 g of sludge powder were dispersed in 50 mL of deionized water, shaken for 30 min and filtered over Extra Rapida-Perfecte 2 (Gruppo Cordenons, Milan, Italy) filter papers. Filtered samples were analysed with a Coulter 360 (Beckman, Irvine, CA, USA) pH-meter. The sample conductivity was tested at 25 °C with a CDM210 (MeterLab, Terni, Italy) conductivity meter.

2.6. Total Organic Carbon and Total Nitrogen Determination

A sample of 0.8 mg sludge powder was put inside a small tin capsule and weighted with a precision scale. The tin capsules were then pressed to remove all the air inside and placed in the instrument autosampler. The elemental C and N quantification was carried out with a CHNS-O Flash 2000 Elemental Analyzer (Thermo-Fisher Scientific, Waltham, MA, USA).

2.7. Plant Growth Parameters

To assess plant growth conditions, data on height (H, cm), shoot biomass (B, g dw) and number of leaves (L) were collected for 5 plants of each species in every treatment. Data were collected at the end of the 8 weeks cultivation period (Supplementary Figure S2). All parameters were combined in the index of plant vigorousness (PL_VIG), calculated as follows:

$$PL\_VIG_{sample\ A} = [(B_{sample\ A}/mean\ B_{Control}) + (H_{sample\ A}/mean\ H_{Control}) + (L_{sample\ A}/mean\ L_{Control})]/3 \quad (1)$$

PL_VIG values < 1 indicated a lower plant performance on SS than under control conditions, conversely values >1 indicated a better plant performance on SS compared to control treatments.

The bioaccumulation factor (BAF) and translocation factor (TF) were calculated as follows:

$$BAF = shoot\ metal\ concentration/sludge\ metal\ concentration \quad (2)$$

$$TF = shoot\ metal\ concentration/root\ metal\ concentration \quad (3)$$

2.8. Statistical Analysis

Five biological replicates each for SS before and after the remediation process and 5 biological replicates of roots and shoots for each plant species were analysed. Resulting data were then organized in 4 datasets (Supplementary Tables S1–S4).

All plant and SS variables were tested for homogeneity using the Levene's test for homogeneity of variance and for normality using the Shapiro-Wilk normality test from the package car (https://cran.r-project.org/web/packages/car/index.html (accessed on 6 July 2021)). For parametric data, ANOVA was performed followed by a Tukey HSD test, while for non-parametric data, a Kruskal-Wallis rank sum test was performed followed by the Dunn's test for multiple comparisons using rank sums (https://cran.r-project.org/web/packages/dunn.test/index.html (accessed on 6 July 2021)) to evaluate the differences among compared groups. To compare single parameters (i.e., pH, EC, etc.) before and after phytoremediation, the Student's *t*-test (parametric data) and the Wilcoxon rank-sum and signed-rank tests (non-parametric data) were used ($p < 0.05$). Statistical and graphical analyses were carried out using R 4.0.2 software and Microsoft Excel© for Microsoft 365.

3. Results

3.1. Sewage Sludge Physico-Chemical Characterization

The physical and chemical properties of analysed sewage sludge (SS) were highly heterogeneous, as a direct consequence of the sewage network served by each wastewater treatment plant (WWTP) and the different final stabilization processes (Table 1, complete dataset in Table S1). Total organic carbon (TOC), N and P percentages ranged between 28–33%, 4.9–6.2%, and 2.2–2.7%, respectively. TOC and N contents (Table 1) were higher in SS from WWTPs performing aerobic digestion as stabilization method (P3, P4, P5 and P6, average TOC 31%, N 6.0%), compared to those without (P1 and P2, average TOC 27%, N 4.7%) ($p < 0.05$). No significant differences were, instead, detected among digested and undigested sludge with respect to other analysed parameters (Table 1).

Table 1. Average physico-chemical characterization of SS from different WWTPs and control soil before remediation. Different letters indicate significant differences among samples for each analysed parameter (ANOVA/Kruskal-Wallis tests, followed by Tukey HSD/Dunn's tests, $p < 0.05$). TOC: total organic carbon, EC: electrical conductivity, DM: dry matter. Complete dataset in Table S1.

Parameter	Control	P1	P2	P3	P4	P5	P6
pH	7.2 ± 0.5^{a}	6.7 ± 0.2^{b}	6.8 ± 0.3^{a}	7 ± 0.3^{a}	6.5 ± 0.2^{b}	6.8 ± 0.3^{a}	7.0 ± 0.2^{a}
EC (dS/m)	0.7 ± 0.2^{a}	2.8 ± 0.3^{b}	2.8 ± 0.5^{b}	2.7 ± 0.1^{b}	2.5 ± 0.2^{b}	2.6 ± 0.3^{b}	3.1 ± 0.2^{c}
DM (%)	30.9 ± 0.8^{a}	26.9 ± 0.8^{b}	25 ± 1^{b}	16.4 ± 0.6^{c}	29 ± 3^{a}	24 ± 4^{b}	20 ± 1^{c}
N (%)	0.59 ± 0.07^{a}	4.9 ± 0.1^{b}	4.5 ± 0.6^{b}	6.5 ± 0.5^{c}	5.9 ± 0.4^{c}	6 ± 1^{c}	6.2 ± 0.7^{c}
TOC (%)	27 ± 2^{a}	28 ± 1^{a}	28 ± 2^{a}	33 ± 2^{b}	33 ± 2^{b}	32 ± 6^{b}	33 ± 3^{b}
P (%)	0.06 ± 0.01^{a}	2.7 ± 0.2^{b}	2.5 ± 0.1^{b}	3.1 ± 0.1^{b}	2.5 ± 0.1^{b}	2.3 ± 0.1^{c}	2.2 ± 0.1^{c}

PH values were close to neutrality or slightly acidic (average of pH 6.8). Electrical conductivity (EC) was comparable in all SS samples (average 2.7 dS/m) except for P6 (3.1 dS/m, $p < 0.05$), while dry matter (DM) content was on average 23.5% (*w*/*w*), apart from P3 and P6 sludge which had a higher water content (average DM 18.2%, $p > 0.05$).

Phosphorus content was similar in P1, P2, P3 and P4 (average 2.7%), but lower in P5 and P6 SS (2.3%, $p < 0.05$). Compared to SS, control soil was characterized by a similar pH, and TOC and DM content, while EC, N, and *p* values were 4, 6, and 40 times lower, respectively.

Sewage sludge total metal concentration ranged between 4.0–6.6 mg/kg dw for As, 0.60–2.4 mg/kg dw for Cd, 68–2450 mg/kg dw for Cr, 250–653 mg/kg dw for Cu, 36–766 mg/kg dw for Ni, 42–114 mg/kg dw for Pb, 3.1–6.9 mg/kg dw for Se and 502–1901 mg/kg dw for Zn (Table 2, complete dataset in Table S1). P3 sludge was more than one order of magnitude higher in Cr and Ni levels compared to the other samples (Table 2). Particularly high values were also detected for other metals in SS from the two biggest WWTPs (P1 and P2) compared to the others: on average 628 mg/kg dw Cu in P1 and P2 samples ($p < 0.05$), and 114 mg/kg dw Pb in P2 ($p < 0.05$).

The bioavailable metal fraction (the part available for plant uptake), ranged between 0–66% of the total metal fraction for Cd, 0.08–0.88% for Cr, 18–34% for Cu, 23–42% for Ni, 20–54% for Pb and 38–56% for Zn. On the whole, Cr was the least available metal to plants (average 0.46%), while Zn appeared to be the most bioavailable (average 44%). Bioavailable fractions of As and Se resulted always below the detection limit.

Table 2. Average metal total concentrations and bioavailable fractions in SS from different WWTPs and control soil before remediation. Small and capital letters indicate significant differences within, respectively, total contents and bioavailable fractions of each specific metal among different SS samples and control soil (Kruskal-Wallis test, followed by the Dunn's test, $p < 0.05$). LOD, limit of detection. Complete dataset in Table S1.

Metal	Control		P1		P2		P3		P4		P5		P6	
	Tot (mg/kg)	Bioavailable Fraction (%)	Tot (mg/kg)	Bioavailable Fraction (%)	Tot (mg/kg)	Bioavailable Fraction (%)	Tot (mg/kg)	Bioavailable Fraction (%)	Tot (mg/kg)	Bioavailable Fraction (%)	Tot (mg/kg)	Bioavailable Fraction (%)	Tot (mg/kg)	Bioavailable Fraction (%)
As	3.3 ± 0.4 ^{a}	<LOD	4.5 ± 0.4 ^{b}	<LOD	6.4 ± 0.3 ^{c}	<LOD	4.6 ± 0.5 ^{b}	<LOD	4.0 ± 0.1 ^{b}	<LOD	6.6 ± 0.8 ^{c}	<LOD	4.4 ± 0.5 ^{b}	<LOD
Cd	1.0 ± 0.2 ^{a}	66 ± 11 ^{A}	2.4 ± 0.1 ^{b}	14 ± 4 ^{B}	1.4 ± 0.1 ^{b}	19 ± 2 ^{B}	0.60 ± 0.01 ^{a}	<LOD	0.92 ± 0.04 ^{a}	<LOD	0.74 ± 0.09 ^{a}	1.9 ± 0.9 ^{C}	0.70 ± 0.08 ^{a}	29 ± 5 ^{B}
Cr	53 ± 1 ^{a}	0.08 ± 0.02 ^{A}	100 ± 6 ^{b}	0.4 ± 0.2 ^{B}	98 ± 1 ^{b}	0.88 ± 0.04 ^{B}	2450 ± 31 ^{c}	0.11 ± 0.05 ^{A}	115 ± 5 ^{b}	0.3 ± 0.1 ^{B}	170 ± 65 ^{b}	0.9 ± 0.7 ^{B}	68 ± 16 ^{a}	0.6 ± 0.2 ^{B}
Cu	27 ± 1 ^{a}	19 ± 4 ^{A}	603 ± 32 ^{b}	23 ± 4 ^{A}	653 ± 11 ^{b}	29 ± 2 ^{B}	250 ± 10 ^{c}	34 ± 4 ^{B}	285 ± 6 ^{c}	18 ± 5 ^{A}	386 ± 50 ^{b}	29 ± 5 ^{B}	321 ± 14 ^{b}	31 ± 10 ^{B}
Ni	26 ± 3 ^{a}	23 ± 3 ^{A}	88 ± 5 ^{b}	40 ± 3 ^{B}	61 ± 1 ^{b}	38 ± 2 ^{B}	766 ± 63 ^{c}	42 ± 7 ^{B}	36.0 ± 0.6 ^{a}	23 ± 1 ^{A}	88 ± 33 ^{b}	40 ± 5 ^{B}	40 ± 2 ^{a}	32 ± 3 ^{C}
Pb	42 ± 4 ^{a}	54 ± 2 ^{A}	85 ± 3 ^{b}	22 ± 4 ^{B}	114 ± 12 ^{c}	27 ± 1 ^{C}	41.6 ± 0.2 ^{a}	27 ± 2 ^{C}	75 ± 3 ^{b}	20 ± 2 ^{B}	45 ± 7 ^{a}	23 ± 5 ^{B}	46 ± 11 ^{a}	31 ± 5 ^{C}
Se	0.07 ± 0.02 ^{a}	<LOD	6.9 ± 0.2 ^{b}	<LOD	3.8 ± 0.3 ^{c}	<LOD	3.3 ± 0.2 ^{c}	<LOD	3.1 ± 0.2 ^{c}	<LOD	4.9 ± 0.5 ^{b}	<LOD	5.3 ± 0.7 ^{b}	<LOD
Zn	120 ± 14 ^{a}	56 ± 9 ^{A}	1263 ± 34 ^{b}	41 ± 3 ^{B}	1403 ± 8 ^{b}	38 ± 4 ^{B}	1128 ± 205 ^{b}	45 ± 2 ^{A}	1901 ± 74 ^{d}	38 ± 6 ^{B}	502± 44 ^{c}	42 ± 4 ^{B}	686 ± 42 ^{c}	46 ± 4 ^{A}

3.2. Plant Growth Performance on Sewage Sludge

Four different common crop plants (*B. juncea*, *B. napus*, *H. annuus* and *Z. mays*) were grown for 8 weeks under controlled conditions on pure SS derived from six different WWTPs (Supplementary Figure S2), and on control soil. These crops were selected for their simple growth requirements and high tolerance to a wide range of soil conditions, making them the most appropriate candidates to grow on sludge and to assess their potential for phytoextraction approach.

Data confirmed that the selected crop plants can be successfully grown on SS, however not all types of sludge supported a similar plant growth performance when compared to the control treatment: 46% of the tested SS resulted in a lower plant vigorousness (PL_VIG, Equation (1)) than the control (PL_VIG < 1), 17% showed a level of plant growth comparable with the control (PL_VIG~1), while 37% supported a higher plant vigorousness than the control (PL_VIG > 1) (complete dataset in Table S2). The sludge samples that allowed for maximum plant growth were from P2 and P4 (average PL_VIG in the 4 species 2.2), while the less suitable SS derived from P1 (average PL_VIG in the 4 species 0.64). The analysed parameters (pH, EC, total metals, bioavailable metals, etc.) were not sufficient to infer a direct relation between sludge characteristics and plant growth performances. Nevertheless, based on our experimental observations, poor plant growth was mainly linked to the low porosity and compact texture of some types of sludge which caused root rotting.

B. juncea (Figure 1a) and *B. napus* (Figure 1b) showed the best performances, with a PL_VIG index increase of 5-fold and 3.5-fold, when grown, respectively, on P4 and P2 sludge. On the other hand, *H. annuus* and *Z. mays* (Figure 1c,d) poorly benefitted from growing on SS, with only a 0.5-fold PL_VIG increase of *H. annuus* grown on P3 sludge ($p < 0.05$), while PL_VIG values of *Z. mays* were either comparable to the control, or significantly lower ($p < 0.05$), such as those grown on P4, P5 and P6.

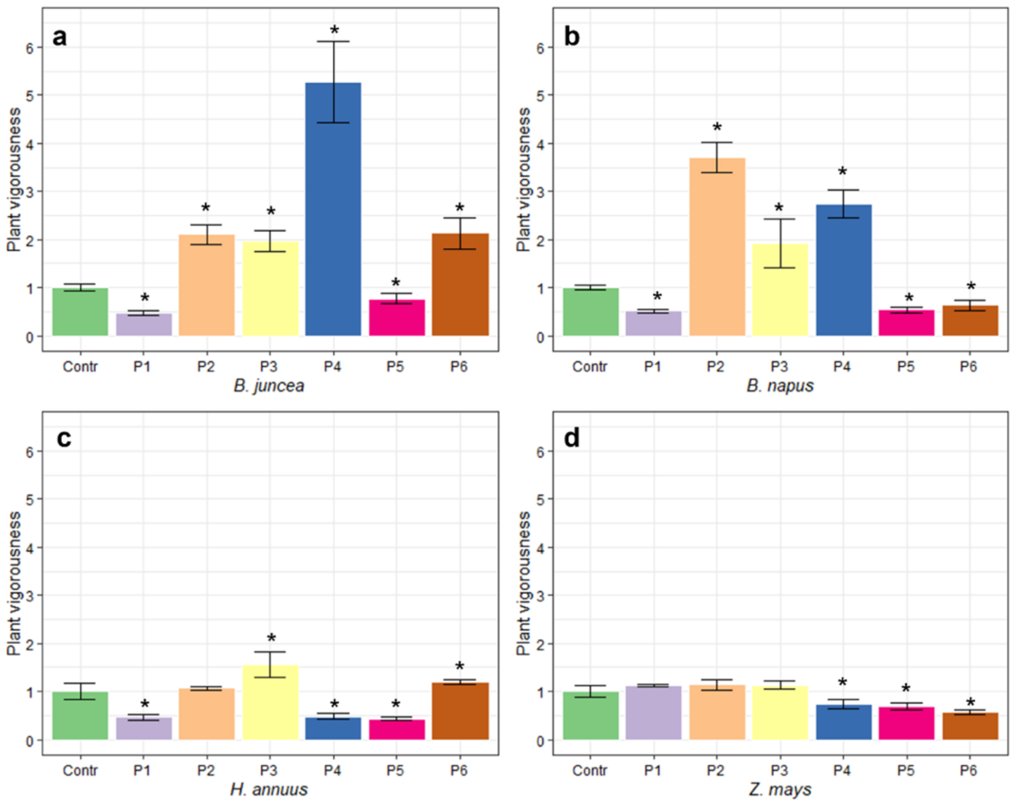

Figure 1. Index of plant vigorousness of (**a**) *B. juncea*, (**b**) *B. napus*, (**c**) *H. annus* and (**d**) *Z. mays* grown on SS from different WWTPs. Stars above each column indicate samples significantly different from the control (T/Wilcoxon tests, $p < 0.05$). Complete dataset in Supplementary Table S2.

3.3. Species Efficiency in Metal Uptake

Metal uptake was highly variable among species, as well as within the same species grown on different substrates (Table 3, complete dataset in Table S2). In fact, when tested with ANOVA all species showed a similar uptake of the different metals (As: $p = 0.6$, Cd: $p = 0.946$, Cr: $p = 0.57$, Cu: $p = 0.996$, Ni: $p = 0.242$, *Pb: N.Q.*; Se: $p = 0.565$, and Zn: $p = 0.693$).

The average shoot metal concentration in the four species was: 0.4 mg/kg dw for As, 0.15 mg/kg dw for Cd, 0.43 mg/kg dw for Cr, 16.5 mg/kg dw for Cu, 10 mg/kg dw for Ni, 0.8 mg/kg dw for Se and 181 mg/kg dw for Zn. Pb concentrations were below the detection limit (Table S2).

B. juncea accumulated significant amounts of Zn, Ni and Cu in the shoots, with average values of 173 mg/kg dw, 15 mg/kg dw and 15 mg/kg dw, respectively. Similar average concentrations of Zn (223 mg/kg dw) were also found in *B. napus*. The average Zn and Cd concentration in *Z. mays* shoots were 147 mg/kg dw and 0.2 mg/kg dw, respectively (Table 3). *H. annuus* accumulated an average of 0.5 mg/kg dw and 182 mg/kg dw, for As and Zn, respectively (Table 3).

Phytoextraction efficiency of each species was assessed through the calculation of translocation factor (TF, Equation (1)) and bioaccumulation factor (BAF, Equation (2)) values (Table 3, complete dataset in Supplementary Table S3). From the comparison of the BAFs and TFs of the tested species, *B. juncea* resulted to be one of the most efficient in metal bioaccumulation with the highest BAFs and TFs for Zn (0.2 and 1), Ni (0.4 and 1) and Cd (0.12 and 1) (Table 3).

B. napus resulted the most efficient species for Zn removal (BAF 0.4, TF 2, Table 3). Despite being the species with the largest biomass, both *Z. mays* and *H. annuus* showed lower BAF values (average for all metals 0.1) compared to *B. napus* and *B. juncea* (0.64 and 0.48, respectively) (Table 3).

3.4. Effects of Phytoremediation on Physico-Chemical Parameters

After 8 weeks of cultivation, the remediated SS was again tested for pH, EC, and TOC, N, P and DM content and the results were compared with those of the initial untreated SS (Figure 2, complete datasets Tables S1 and S4). Controls were tested likewise. Soil pH (Figure 2a) did not exhibit significant variations, except for P1 and P2 sludge, which resulted more acid after remediation than before (0.3 and 0.7 pH decrease, respectively), and P4 sludge, whose pH conversely increased (+0.5) compared to the corresponding raw sludge. Electrical conductivity (Figure 2b) generally increased of about the 25% after the remediation process, in particular, the values for P1, P4 and P5 passed from 2.6 to 3.5 dS/m ($p < 0.01$). Sludge DM content (Figure 2c) increased 10% on average, with peaks of 20% and 21% in P2 and P6 sludge, respectively ($p < 0.01$). TOC, N and P content showed a decrease after plant growth (−7%, −20% and −12% on average, respectively). Finally, phosphorus content (Figure 2f) decreased on average from 2.5% to 2.1% ($p < 0.01$) in after SS remediation.

3.5. Metal Removal from Sewage Sludge

The amount of each metal removed from different types of SS by the four tested plant species was calculated as the difference between the total amount of metal present in raw sludge (8 kg of SS in each box) and the total amount of metal accumulated in the aboveground plant biomass (considering the total biomass of 11 plants produced in each box) (Table S2). The data were expressed as percentages of the initial concentrations (Table S1) in SS (Table 4).

Plant biomass production was a key factor in phytoremediation efficiency of the different crop species. The best results were obtained for P2, P3 and P4 sludge on which plants, in general, grew bigger and the average metal removal was 1.4%, 0.58% and 0.64%, respectively, of the total metal content. The average percentage of each metal removed by the selected crops was 0.20% for As, 0.85% for Cd, 0.09% for Cr, 0.36% for Cu, 0.36% for Ni, 3.9% for Se and 1.2% for Zn, with respect to the initial average amount.

Table 3. Average shoot total metal concentration (mg/kg), metal bioaccumulation (BAF) and translocation (TF) factors in the four analysed plant species. Different letters indicate significant differences for the three different parameters among species (ANOVA/Kruskal-Wallis tests followed by Tukey HDS/Dunn's test, $p < 0.05$). Total Pb level in shoots was below the detection limit and is not reported here as well as the related BAF and TF values. Complete datasets in Tables S2 and S3.

	As			Cd			Cr			Cu			Ni			Se			Zn		
Species	**Conc.**	**BAF**	**TF**	**Conc.**	**BAF**	**TF**	**Conc.**	**BAF**	**TF**	**Conc.**	**BAF**	**TF**	**Conc.**	**BAF**	**TF**	**Conc.**	**BAF**	**TF**	**Conc.**	**BAF**	**TF**
H. annuus	0.5 ± 0.3^{a}	0.2 ± 0.1^{A}	0.40 ± 0.2^{x}	0.1 ± 0.1^{a}	0.13 ± 0.06^{A}	1.5 ± 0.9^{x}	0.5 ± 0.5^{a}	<LOD	2 ± 1^{x}	15 ± 8^{a}	0.06 ± 0.04^{A}	0.8 ± 0.8^{x}	9 ± 9^{a}	0.04 ± 0.03^{A}	1 ± 1^{x}	0.7 ± 0.4^{a}	0.2 ± 0.2^{A}	3 ± 2^{x}	182 ± 80^{a}	0.2 ± 0.2^{A}	2 ± 1^{x}
B. juncea	0.5 ± 0.2^{a}	0.09 ± 0.05^{A}	0.6 ± 0.4^{x}	0.1 ± 0.1^{a}	0.12 ± 0.04^{A}	1 ± 1^{x}	0.3 ± 0.3^{a}	<LOD	0.6 ± 0.6^{x}	15 ± 8^{a}	0.06 ± 0.04^{A}	1.3 ± 0.9^{x}	15 ± 7^{a}	0.4 ± 0.2^{B}	1 ± 1^{x}	0.8 ± 0.3^{a}	2 ± 2^{A}	3 ± 3^{x}	173 ± 60^{a}	0.2 ± 0.1^{A}	1.0 ± 0.8^{y}
Z. mays	0.3 ± 0.2^{a}	0.03 ± 0.03^{A}	0.3 ± 0.3^{x}	0.2 ± 0.2^{a}	0.16 ± 0.3^{A}	2.0 ± 0.7^{x}	0.7 ± 0.5^{a}	0.01 ± 0.01^{A}	2 ± 2^{x}	18 ± 10^{a}	0.06 ± 0.03^{A}	1.6 ± 0.8^{y}	7 ± 5^{a}	0.05 ± 0.02^{A}	1.1 ± 0.6^{x}	0.8 ± 0.4^{a}	0.3 ± 0.1^{A}	1.0 ± 0.2^{x}	147 ± 70^{a}	0.2 ± 0.1^{A}	2 ± 2^{x}
B. napus	0.3 ± 0.2^{a}	0.03 ± 0.03^{A}	0.1 ± 0.1^{x}	0.2 ± 0.24^{a}	0.3 ± 0.3^{A}	1 ± 1^{x}	0.2 ± 0.2^{a}	<LOD	0.2 ± 0.2^{x}	18 ± 11^{a}	0.07 ± 0.04^{A}	2 ± 1^{y}	9 ± 6^{a}	0.04 ± 0.03^{A}	1.2 ± 0.9^{x}	0.9 ± 0.4^{a}	3 ± 3^{A}	0.8 ± 0.4^{x}	223 ± 140^{a}	0.4 ± 0.3^{B}	2 ± 1^{x}

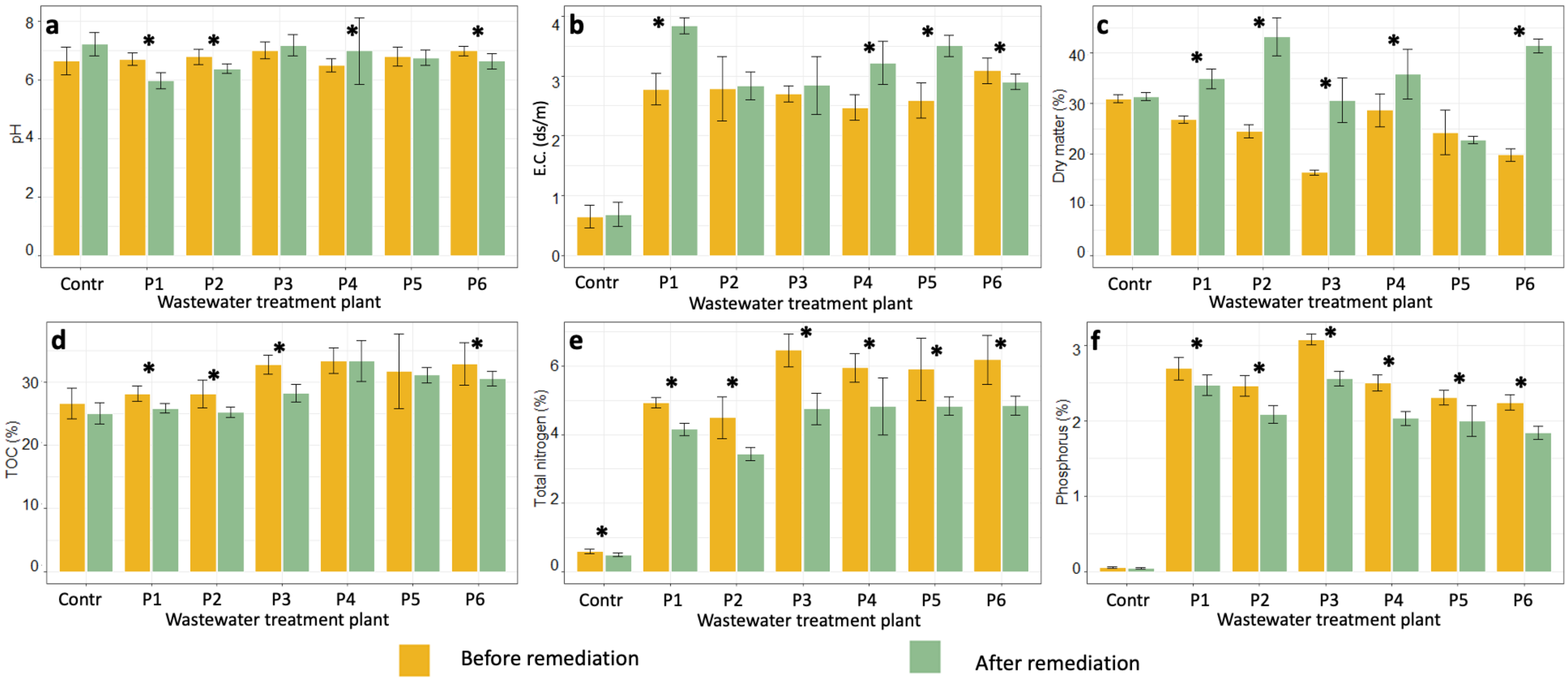

Figure 2. Effects of phytoremediation on SS physical and chemical characteristics. (**a**) pH; (**b**) electrical conductivity (EC); (**c**) dry matter content; (**d**) total organic carbon (TOC); (**e**) total nitrogen; (**f**) phosphorus. Each value is the average of 5 replicates ($n = 5$). Stars above each couple of columns identify significant differences before and after the remediation process (*t*-test, $p < 0.05$). Complete datasets in Supplementary Tables S1 and S4.

Table 4. Metal removal after phytoremediation. Values are the average of 5 replicates (n = 5). WWTP, wastewater treatment plant; LOD, limit of detection. Stars indicate significant differences in metal removal (%) with respect to the control, within the same species (T/Wilcoxon test, $p < 0.05$). Complete dataset in Supplementary Table S4.

WWTP	Species	As (%)	Cd (%)	Cr (%)	Cu (%)	Ni (%)	Pb (%)	Se (%)	Zn (%)
Control	*H. annuus*	0.05 ± 0.01	0.4 ± 0.1	<LOD	0.52 ± 0.04	0.03 ± 0.01	<LOD	2.4 ± 0.6	0.90 ± 0.1
Control	*B. juncea*	0.03 ± 0.01	0.5 ± 0.1	<LOD	0.24 ± 0.02	0.01 ± 0.01	<LOD	22 ± 3	0.57 ± 0.06
Control	*Z. mays*	0.09 ± 0.01	0.19 ± 0.02	0.26 ± 0.08	0.7 ± 0.1	0.35 ± 0.09	<LOD	3.6 ± 0.3	1.2 ± 0.2
Control	*B. napus*	0.05 ± 0.01	0.12 ± 0.02	<LOD	0.38 ± 0.01	0.01 ± 0.01	<LOD	47 ± 10	1.32 ± 0.07
P1	*H. annuus*	0.01 ± 0.01 *	0.08 ± 0.02 *	<LOD	0.05 ± 0.01 *	0.06 ± 0.01 *	<LOD	0.20 ± 0.01 *	0.13 ± 0.01 *
P1	*B. juncea*	0.09 ± 0.02 *	0.05 ± 0.02 *	<LOD	0.01 ± 0.01 *	0.5 ± 0.2	<LOD	0.03 ± 0.01 *	0.05 ± 0.01 *
P1	*Z. mays*	0.30 ± 0.03 *	2.7 ± 0.25 *	0.09 ± 0.03	0.62 ± 0.08	0.33 ± 0.06	<LOD	1.6 ± 0.2 *	1.8 ± 0.2
P1	*B. napus*	<LOD	0.11 ± 0.01	<LOD	0.03 ± 0.01 *	0.04 ± 0.01 *	<LOD	0.10 ± 0.01 *	0.18 ± 0.01 *
P2	*H. annuus*	0.04 ± 0.01	0.11 ± 0.01 *	0.01 ± 0.01	0.14 ± 0.01 *	0.3 ± 0.1 *	<LOD	1.2 ± 0.2	0.40 ± 0.03 *
P2	*B. juncea*	2.1 ± 0.21 *	0.17 ± 0.01 *	<LOD	0.14 ± 0.01 *	0.25 ± 0.06 *	<LOD	1.9 ± 0.4 *	1.0 ± 0.1 *
P2	*Z. mays*	0.09 ± 0.01	0.30 ± 0.01 *	<LOD	0.23 ± 0.02 *	0.40 ± 0.04	<LOD	3.1 ± 0.4	1.3 ± 0.1
P2	*B. napus*	0.16 ± 0.01 *	<LOD	0.03 ± 0.01	0.31 ± 0.01	0.16 ± 0.05	<LOD	1.4 ± 0.4	1.27 ± 0.05 *
P3	*H. annuus*	0.20 ± 0.02 *	2.0 ± 0.1 *	0.01 ± 0.01	1.0 ± 0.5	1.3 ± 0.3 *	<LOD	4.4 ± 0.8 *	3 ± 2
P3	*B. juncea*	0.10 ± 0.01 *	3.5 ± 0.8 *	<LOD	1.1 ± 0.5 *	0.9 ± 0.2 *	<LOD	2.6 ± 0.3 *	3 ± 1
P3	*Z. mays*	0.09 ± 0.01	<LOD	<LOD	0.39 ± 0.04 *	0.43 ± 0.09	<LOD	2.1 ± 0.4 *	1.4 ± 0.3
P3	*B. napus*	0.18 ± 0.02 *	3.1 ± 0.5 *	<LOD	0.91 ± 0.08 *	0.97 ± 0.06 *	<LOD	4.0 ± 0.3 *	4.4 ± 0.8 *
P4	*H. annuus*	0.21 ± 0.05 *	0.4 ± 0.2	0.02 ± 0.01	0.14 ± 0.04 *	0.05 ± 0.03	<LOD	0.48 ± 0.05 *	0.5 ± 0.2
P4	*B. juncea*	0.22 ± 0.02 *	0.65 ± 0.01	0.18 ± 0.06	0.83 ± 0.04 *	0.97 ± 0.44	<LOD	4.8 ± 0.5 *	1.5 ± 0.2 *
P4	*Z. mays*	0.04 ± 0.01 *	0.5 ± 0.2	<LOD	0.26 ± 0.01 *	0.33 ± 0.04	<LOD	1.1 ± 0.2 *	0.29 ± 0.01 *
P4	*B. napus*	0.13 ± 0.02 *	0.39 ± 0.03 *	<LOD	0.44 ± 0.02	0.35 ± 0.07 *	<LOD	2.4 ± 0.9 *	0.77 ± 0.05 *
P5	*H. annuus*	0.8 ± 0.2	0.24 ± 0.09	<LOD	0.10 ± 0.05 *	0.09 ± 0.07	<LOD	0.34 ± 0.06 *	0.80 ± 0.09
P5	*B. juncea*	0.01 ± 0.01	<LOD	<LOD	0.07 ± 0.01 *	1.0 ± 0.5	<LOD	0.09 ± 0.02 *	0.14 ± 0.03 *
P5	*Z. mays*	0.01 ± 0.01 *	<LOD	<LOD	0.10 ± 0.01 *	0.07 ± 0.01 *	<LOD	0.35 ± 0.02 *	0.8 ± 0.2
P5	*B. napus*	0.01 ± 0.01 *	0.21 ± 0.09	<LOD	0.04 ± 0.01 *	0.02 ± 0.01	<LOD	0.13 ± 0.01 *	0.45 ± 0.04 *
P6	*H. annuus*	<LOD	1.3 ± 0.3 *	0.23 ± 0.03	0.33 ± 0.02 *	0.18 ± 0.03 *	<LOD	0.9 ± 0.5 *	1.6 ± 0.2 *

Table 4. *Cont.*

WWTP	Species	As (%)	Cd (%)	Cr (%)	Cu (%)	Ni (%)	Pb (%)	Se (%)	Zn (%)
P6	*B. juncea*	0.08 ± 0.02	0.34 ± 0.01	0.04 ± 0.02	0.45 ± 0.09	0.5 ± 0.1 *	<LOD	1.1± 0.1 *	1.6 ± 0.1 *
P6	*Z. mays*	0.40 ± 0.07 *	1.6 ± 0.2 *	0.05 ± 0.02 *	0.24 ± 0.03 *	0.16 ± 0.04	<LOD	0.8 ± 0.2 *	0.5 ± 0.2 *
P6	*B. napus*	0.15 ± 0.04 *	1.1 ± 0.2 *	0.02 ± 0.01	0.13 ± 0.01 *	0.11 ± 0.03 *	<LOD	0.27 ± 0.05 *	0.5 ± 0.1 *

Se and Zn were most efficiently extracted. Se removal was particularly high from the control substrate (47% removed by *B. napus*) and from P3 sludge (around 4.2% removed by *H. annuus* and *B. napus*), whereas Zn was removed up to 4.4% by *B. napus* growing on P3 sludge. *B. juncea* and *B. napus* proved to be the best species for the removal of Se (1.7% and 1.4%, respectively), Zn (1.3% and 1.3%, respectively) and Cu (0.43% and 0.31%, respectively). *B. juncea* also removed 3.5% of Cd and 2.1% of As from P3 and P2 sludge, respectively. On the other hand, Cr and As were poorly extracted by all the tested species (0.08% and 0.20%, of the total, respectively) (Table 4).

4. Discussion

The physico-chemical properties of the analysed sludges were in line with the values reported by Cěrne et al. [37], who found 36% TOC, 5.7% N and 2.6% P in municipal sludge samples collected in Croatia in several wastewater treatment plant (WWTP). Our results showed a general decrease in TOC and N, in SS that undergone anaerobic digestion (Table 1). In accordance with these data, Yoshida et al. [38] reported losses in N and TOC content up to 23% in anaerobically digested sludge compared with unstabilized ones. Lower TOC, N, P and dry matter (DM) contents have also previously been detected in sludge treated with anaerobic stabilization (29%, 4.2%, 23.7%, 23.9%, respectively) compared with those aerobically stabilized (43%, 7.2%, 28.1%, 33.6%, respectively) [37].

The measured average concentrations of Cd, Cu and Zn were in line with the values reported for the main WWTPs in Europe (Cd 2.1 mg/kg dw, Cu 340 mg/kg dw, Zn 1071 mg/kg dw) [39]. Considering the legal limits for nutrients and toxic metals [14], all analysed SS was suitable for agricultural use, except for P3 sludge, which showed Cr and Ni concentration respectively 8 and 3 times above the limits (Table 2). In this sludge the high Ni and Cr concentrations are the direct consequence of the galvanic industries present in the area served by the WWTP. A similar result was previously reported for the Shanxi region in China [17]. The enrichment of Cu and Pb reported for P1 and P2 sludges was probably connected to the large urban areas served by these plants, as these metals typically derive from urban street dust [16].

The bioavailable metal fraction of some elements in sludges was substantially low (i.e., Cr 0.46%, Cd 19%, Cu 25%) due to complexation with the large quantity of organic matter present in SS and the slightly alkaline pH. This effect was previously reported for mine soils (Madrid, Spain) enriched in the same elements, amended with sheep/horse manure, wood compost, *Sphagnum* peat and pine bark [40]. These opposite responses have been already documented by other studies [41–43], in which plants (*Catharanthus roseus* G. Don, *B. napus*, *Alocasia macrorrhiza* L. and *Sedum alfredii* (Hance) cultivated on pure SS showed enhanced growth compared to those grown on control soil, due to the abundance of organic matter, N, P and other micronutrients, such as Mn, Cu, and Zn. On the other hand, SS can also suppress plant growth due to its toxicity, as was reported by Suchkova et al. [44], who did not record any natural plant species (i.e., *Amaranthus albus* L., *Cynodon dactylon* (L.) Pers., *Portulaca oleracea* L.) growing on 100% municipal sludge from Sindos (Greece), compared to sludges from other WWTPs. The 3-fold higher PL_VIG found of the *Brassica* species (on average) compared to *H. annuus* and *Z. mays* (especially in P2 and P4 sludges) can be ascribable to their higher efficiency in using NH_4^+ (abundant in SS) as source of nitrogen [45].

Regarding metal accumulation, *B. juncea* accumulated significant amounts of Zn, Ni and Cu in the shoots, (average values of 173 mg/kg dw, 15 mg/kg dw and 15 mg/kg dw) and these concentrations were in line with Zn and Ni concentrations (around 180 mg/kg dw and 30 mg/kg dw, respectively) detected for the same species grown on agricultural soils polluted by long-term sewage sludge application in Pietermaritzburg, South Africa [46]. The average Zn and Cd concentration in *Z. mays* shoots (147 mg/kg dw and 0.2 mg/kg dw, respectively, Table 3) were in line with values reported by Xu et al. [47] (147 mg/kg dw and 0.3 mg/kg dw, respectively), who cultivated this species on 80% SS from the Datansha WWTP (Guangzhou, China) mixed with 20% soil.

H. annuus has been indicated as a promising species for As and Zn sludge remediation, achieving concentration of 3.2 mg/kg dw and 400 mg/kg dw respectively, in shoots [29]. However, in the present study this species was poorly efficient in uptaking As and Zn reaching the 16% and the 50 % respectively (Table 3), of the reported SS concentrations [29]. However, the most efficient species for As removal has been identified in *Pteris vittata* [48] which has never been tested on SS. *B. juncea* resulted the most efficient species in metal bioaccumulation of Zn, Ni and Cd, our results showed a better capacity of this species to accumulate metals compared to previously reported data which showed significantly lower values of BAF (0.02, 0.05, 0.08) and TF (0.15, 0.37, 0.52) for the previously cited metals [30].

B. napus was similarly efficient to the previous species for Zn removal (BAF 0.4, TF 2), analogous performances were documented by Brunetti et al. [49], growing this plant on contaminated soil mixed with 10% compost (BAF 0.3 and TF 2.4), confirming the high Zn uptake capacity of this species.

After 8 weeks, remediated sludge showed a strong volume reduction, while texture and structure resulted highly improved. In fact, after phytoremediation SS was less compact with a higher porosity in comparison to raw SS. The increased conductivity (+25%) of SS after remediation can be ascribed to the degradation of organic substances and consequent release of minerals. Regarding TOC, N and P all values decreased of the 7%, 20% and 12% on average, but remained above the minimum law requirements (TOC > 20 %, N > 1.5 %, P > 0.4 %; [14]) for agricultural reuse. The removal of metals was in general low (0.1–3.9%) with some exception as for example Zn removal by *B. napus* on P3 sludge (4.4%). Analogous results were found by Brunetti et al. [49], who reported a Zn extraction rate of 4.5%, cultivating *B. napus* in similar conditions, thus confirming the potential of this plant for Zn phytoremediation. Because of the low extraction rate of Cr and Ni in SS (max 0.23 % for Cr and 1.3% for Ni), the residual concentration of these metals in P3 sludge (2376 mg/kg dw and 729 mg/kg dw, respectively) still exceeded the law limit for these two pollutants (<300 mg/kg for Ni, <200 mg/kg for Cr, [15]), not allowing its reuse in agriculture. However, since no plants have ever been reported accumulating high level of Cr, its removal is difficult to achieve through phytoremediation [48].

5. Conclusions

Our results show that *Z. mays*, *H. annuus*, *B. napus* and *B. juncea* crop plants can be successfully grown on pure SS and have the ability to accumulate small quantities of metals while ameliorating the physical properties of this matrix. The highest levels of metal removal (more than 4% for Se and Zn) have been achieved by *B. juncea* and *B. napus* on P3 and P4 sludge. The amount of extracted metal was however not sufficient to bring the levels of toxic ions (i.e., Ni and Cr in P3 sludges) within law values acceptable for agricultural SS reuse. However, phytoremediation had a positive impact on the physical properties (texture and structure) of the sludge and its volume largely decreased due to the loss of water. Plant growth had a limited impact on decreasing TOC, N and P content, and their concentration in remediated sludge was still largely above the regulatory limits for agricultural use.

In conclusion, even though the percentage of extracted metals were not sufficient to allow its agricultural reuse, this study reports the possibility to use phytoremediation as a viable method to clean-up trace metal contaminated SS.

Future studies will be focused on the cultivation on SS of hyperaccumulator plant species, which will be essential in achieving higher metal removal rates. If successful the application of this new technique may avoid the landfilling/incineration of SS, bringing important financial benefits to wastewater treatment utilities and environmental benefits for the whole society.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11122456/s1, Figure S1: Example of undrilled plastic culture box (40 × 30 × 20 cm). Figure S2: Crop species growing on sewage sludge after 2, 5, and 8 weeks. Table S1: Sewage sludge physico-chemical characteristics before phytoremediation. Table S2: Plant growth parameters and metal uptake. Table S3: Plant bioaccumulation and translocation factors. Table S4: Sewage sludge physico-chemical characteristics after phytoremediation.

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Data Availability Statement: All data reported in the present study are available as Supplementary Materials (Tables S1–S4).

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