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The Application of Different Biological Remediation Strategies to PCDDs/PCDFs Contaminated Urban Sediments

Magdalena Urbaniak [1,2,*], Anna Wyrwicka [3], Grzegorz Siebielec [4], Sylwia Siebielec [4], Petra Kidd [5] and Marek Zieliński [6]

1 Department of Applied Ecology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland
2 European Regional Centre for Ecohydrology of the Polish Academy of Sciences, Tylna 3, 90-364 Lodz, Poland
3 Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland; anna.wyrwicka@biol.uni.lodz.pl
4 Institute of Soil Science and Plant Cultivation—State Research Institute, Czartoryskich 8, 24-100 Pulawy, Poland; gs@iung.pulawy.pl (G.S.); ssiebielec@iung.pulawy.pl (S.S.)
5 Instituto de Investigaciones Agrobiológicas de Galicia (IIAG), Consejo Superior de Investigaciones Científicas (CSIC), Santiago de Compostela 15706, Spain; pkidd@iiag.csic.es
6 Nofer Institute of Occupational Medicine, Teresy 8, 91-348 Lodz, Poland; marekz@imp.lodz.pl
* Correspondence: m.urbaniak@unesco.lodz.pl or magdalena.urbaniak@biol.uni.lodz.pl

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Abstract: Our aim was to assess the efficacy of four different bioremediation strategies applied to soil treated with urban sediments for alleviating soil phytotoxicity (examined using *Lepidium sativum*), by removing polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), and mitigating the toxic effect on plants by the applied sediment: (1) Natural attenuation, (2) phytoremediation with the use of two plants *Tagetes patula* L. and *Festuca arundinacea*, (3) rhizobacterial inoculation with *Massilia niastensis* p87 and *Streptomyces costaricanus* RP92 strains, (4) rhizobacteria-assisted phytoremediation with both plants and strains. The applied sediment had a positive influence on *L. sativum* growth (90% higher than in the unamended soil), mostly due to its high content of nutrients, mainly Ca and Fe, which immobilize pollutants. The positive effect of sediments continued for up to 10-week duration of the experiment; however, the rhizobacterial inoculated samples were characterized by higher growth of *L. sativum*. The application of rhizobacteria-assisted phytoremediation further increased the growth of *L. sativum*, and was also found to improve the efficiency of PCDD/PCDF removal, resulting in a maximum 44% reduction of its content. This strategy also alleviated the negative impact of urban sediments on *T. patula* and *F. arundinacea* biomass, and had a beneficial effect on protein and chlorophyll content in the studied plants.

Keywords: urban sediments; PCDDs/PCDFs; rhizobacterial inoculants; bioremediation; phytoremediation

1. Introduction

The urban water ecosystems, located downstream of city landscapes, often become reservoirs for a variety of pollutants originating from atmospheric deposition, as well as urban runoff, storm water outlets, industrial waste and combined sanitary overflows [1–3]. To minimize the inflow of such pollutants to river ecosystems and their further transport along the river continuum, small dam reservoirs, sedimentation ponds and biofilters might be used. These constructions create ideal conditions for the sedimentation and deposition of particulate matter by decreasing the flow velocity, thus acting as efficient traps for associated compounds of urban origin [4–12]. However, the accelerated

accumulation of sediments and associated pollutants leads to rapid siltation of such constructions, and this requires periodical dredging of the accumulated sediments and their further utilization [7]. These dredged urban sediments are usually stored in landfill areas, where they act as potential hazardous material for the surrounding environment. In addition, this solution creates economic burdens for the municipality due to the need to transport the sediments out of the city.

One promising method of managing the increasing amount of urban reservoir sediments is their direct application within the city limits as soil additives on city gardens and lawns. This type of urban sediment utilization is possible due to their richness in nutrients and organic matter, which improve soil properties and promote plant growth. Nevertheless, urban sediments also contain other compounds with harmful properties, such as heavy metals, pesticides washed out from urban green areas, car oils from streets and parking areas, and a variety of other organic compounds of industrial or anthropogenic origin. One of the most toxic groups of compounds, which have carcinogenic, hepatotoxic, immunotoxic and neurotoxic properties, are polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs) [9,10]. These pollutants are characterized by a wide range of occurrence in the urban water ecosystems, because their main source is the load of domestic and industrial wastewater, atmospheric emission and deposition as well as emission from other sources associated with human activity in the city space such as car traffic [10].

Due to highly hydrophobic characteristics of PCDDs/PCDFs, they undergo rapid deposition in river and reservoir sediments, thus lowering the quality of the urban ecosystem and decreasing its biodiversity. Owing to the toxic properties of PCDDs/PCDFs, their persistence in the environment and their ability to bioaccumulate in aquatic and terrestrial trophic chains, the European Commission classified them as priority hazardous substances in the field of water policy, and imposed a requirement for EU members to monitor and eliminate them from the environment [13].

A potential approach to be applied for inactivation of urban sediments contaminated with PCDDs/PCDFs is phytoremediation—a method aimed at removal or decomposition of the pollutants using plants. Nevertheless, PCDDs/PCDFs due to their hydrophobicity and hence their strong adsorption by sediment and soil particles, transfer to sediment or soil solution to a very small extent. Consequently, the ability of plants to uptake PCDDs/PCDFs from sediments or soil is very limited [14]. In this situation, plants can be used to promote the continued existence of indigenous soil microorganisms, which are able to biodegrade PCDDs/PCDFs (rhizodegradation). In addition, inoculation of these contaminated sediments with appropriately selected specific single strains or consortia of microorganisms may further increase the efficiency of biodegradation processes.

Such remediation approaches as natural bioremediation with indigenous microorganisms (i.e., natural attenuation), inoculation and phytoremediation can be used separately; however, the most promising solution is for their combined use as part of rhizobacteria-assisted phytoremediation, intended to optimize the synergistic effect of selected plants and bacterial strains. Until now, this approach has been successfully used for cleaning soil contaminated with both organic and inorganic compounds [15–18]; however, no such studies have been performed on the remediation of urban sediments used directly within city limits.

The key step in designing an effective rhizobacteria-assisted phytoremediation strategy is the selection of a suitable plant-bacteria partnership to maximize the removal efficiency of a given pollutant [15,19]. Among the plants used in the remediation of contaminated soil, two species are of particular relevance, and are also specific to city gardens and lawns: *Tagetes patula* L. (*Asteraceae*) is commonly used as an ornamental plant, whereas *Festuca arundinacea* Schreb. (*Poaceae*) is often used in reclamation and as a grass species sown on lawns.

Tagetes patula L., commonly known as French marigold, is a robust and non-fussy plant originating from Mexico, used mainly as an edging plant on herbaceous borders. Marigold produces secondary metabolites, which assist in the remediation of combined contaminated sites [20,21]. It also contains bioactive compounds, which are widely employed as insecticides, fungicides and nematicides [22]; while *Festuca arundinacea* Schreb., commonly known as Tall fescue, is an evergreen, tuft-forming grass

with a deep root system. It is a cool-season perennial C3 species native to Europe, frequently used in the phytoremediation of soil contaminated with organic compounds [18,20,23,24].

Among the bacterial strains used for bioremediation, *Streptomyces costaricanus* RP92, isolated from the rhizosphere of *Cytisus striatus*, is especially interesting because it can improve the remediation of soil contaminated with chloroorganic compounds such as hexachlorocyclohexane or diesel oils [25–27]. The strain *Massilia niastensis* P87, isolated from the rhizosphere of *Festuca rubra*, found growing on mine tailings with elevated concentrations of Cd, Pb and Zn [27], would further improve the bioremediation efficiency of the mixtures of pollutants, including trace metals which accumulate in urban sediments.

Considering the above, our aim was to assess and compare the efficacy of four different environmentally friendly strategies in the remediation of soil contaminated with PCDDs/PCDFs by urban bottom sediment application: (1) natural attenuation, (2) phytoremediation with two selected plant species *Tagetes patula* L. and *Festuca arundinacea* Schreb., (3) rhizobacterial inoculation with two selected strains *Streptomyces costaricanus* RP92 and *Massilia niastensis* P87, and (4) rhizobacteria-assisted phytoremediation using both sets of plants and bacterial strains given above. At the same time, the effects of urban sediment application and the said remediation strategies on soil phytotoxicity were evaluated. In addition, the plant biomass, total soluble protein content, total chlorophyll content and chlorophyll a/b ratio were measured to assess plant response to the applied urban sediments and remediation strategies.

2. Materials and Methods

2.1. Urban Sediments and Soils

The urban bottom sediments were collected from a sedimentation zone of the sequential sedimentation-biofiltration system located in Lodz, Poland, on the Sokołówka River. The system was constructed to reduce the inflow of particulate matter and a range of organic, inorganic and bacterial pollutants from storm water coming from the most urbanized catchment area of the Sokołówka River.

The system comprises (1) the hydrodynamically intensified sedimentation zone that facilitate the sedimentation and deposition processes, and in this way enable pre-treatment of the inflowing river and storm water; (2) the intensified biogeochemical processes zone, where fine particles are sieved and nutrients are reduced; and (3) intensified biofiltration zone planted with macrophytes (*Phragmites australis, Typha latifolia* and *Acorus calamus*) responsible for removal of nutrients and organic compounds [28,29].

Fresh sediments, with a dry matter (DM) content of 35%, were mixed with agricultural uncontaminated Haplic Luvisol type soil, in a 1:10 proportion and transferred into 2 kg pots. Prior to mixing, the soil was sieved using a 2-mm sieve.

2.2. Pot Experiment Design

The experimental design included four variants:

1. Natural attenuation—uncontaminated soil was mixed with fresh urban sediments, and no plants or bacterial inoculants were added;
2. Phytoremediation—uncontaminated soil was mixed with fresh urban sediments and *T. patula* L. or *F. arundinacea* Schreb. were planted;
3. Rhizobacterial inoculation—uncontaminated soil was mixed with fresh urban sediments and *S. costaricanus* RP92 or *M. niastensis* P87 were added, no plants were grown;
4. Rhizobacteria-assisted phytoremediation—using both the studied plants and bacterial strains (Figure 1).

All the variants were performed in triplicate (three separate pots per one remediation variant).

T. patula L. was seeded at a rate of 15 seeds per pot, which was reduced to 10 plants per pot after germination. *F. arundinacea* seeds were seeded at a rate of 2 g per pot.

Fresh cultures of bacterial strains were grown in 869 liquid medium [30] for 24 h. Five mL of this pre-culture was then grown for 12 h in fresh 869 liquid medium. Subsequently, bacterial biomass was collected by centrifugation (6000 rpm, 15 min), washed once and re-suspended in 10 mM $MgSO_4$ solutions to an OD_{660} of 1.0 (about 10^7 cells per mL). Each plant pot was inoculated during the germination phase (four weeks after seeding) with 100 mL of bacterial suspension. The same amount of sterile 10 mM $MgSO_4$ was added to non-inoculated pots. The inoculation was repeated after three weeks using the same procedure.

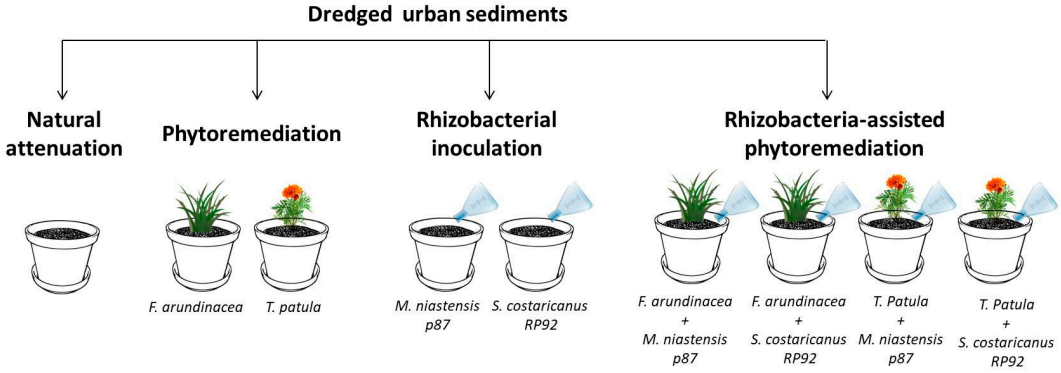

Figure 1. Experiment design.

The pot study was run for a 10-week period in a greenhouse under controlled conditions, i.e., supplemental light and 27 °C/20 °C day/night temperatures. After this time, the plants were harvested, the fresh biomass was weighed and the protein and chlorophyll content, as well as the chlorophyll a/b ratio, were measured in plant green tissues.

After harvesting, soil from each treatment was mixed, sieved to 2-mm sieve to remove the plant residues, and aliquots of soil were collected for further analyses of PCDD/PCDF concentrations and the phytotoxicity bioassay.

2.3. Soil Analyses

2.3.1. Determination of Physico-Chemical Parameters

Soil pH was measured in H_2O using a 1:2.5 soil:solution ratio. Total C was measured by combustion with a CN analyzer (Elementar, vario Macro cube, Langenselbold, Germany). The sediment samples were digested for elemental composition analysis in a 3:1 mixture of concentrated HNO_3:HCl in Teflon PFA vessels in a microwave accelerated reaction system (MarsXpress; CEM Corp., Matthews, NC, USA); total concentrations of elements were analyzed by ICP-MS (Agilent 7500ce, Agilent Technologies Inc., Santa Clara, CA, USA).

2.3.2. Determination of PCDD/PCDF Concentrations

For analysis of the concentrations of 17 toxic congeners of PCDD/PCDF, the PN-EN 1948-3 [31] and US EPA Method 1613 [32] were applied. The analysis was carried out based on isotope dilution and high-resolution gas chromatography (HRGC)/high-resolution mass spectrometry (HRMS) using an HP 6890 N Agilent Technologies GC coupled with a high-resolution mass spectrometer AutoSpec Ultima. The detailed description of the applied analytical procedure is depicted in the work by Urbaniak et al. [8]. The obtained concentrations were calculated using Toxic Equivalency Factor (TEF) and expressed as the Toxic Equivalency (TEQ) [33,34].

Quality assurance/quality control procedure was carried out using certified calibration standards. Each analytical series contained sample blank, control, certified reference material and in-house QC samples. Samples' spikes were used also as an additional check of accuracy. Recoveries of ^{13}C-labeled PCDDs/PCDFs ranged from 74% to 146%. Artefacts were estimated using a reagent blank, and

duplicate analysis enabled to verify the precision that ranged from 2% to 11%. The obtained LOD values ranged from 0.070 to 0.143 ng/kg for PCDDs and 0.042 to 0.137 ng/kg for PCDFs.

2.3.3. Phytotoxicity Analysis

The commercially available bioassay Phytotoxkit^{TM} Test (Microbiotest Inc., Nazareth, Belgium) was used to assess the phytotoxicity of the soil samples [35]. The principle of the test is based on measurement of the inhibition of the length of roots of test species after 3 days of exposure to contaminated soil in relation to a reference soil. For the purpose of this experiment, the dicotyledon *Lepidium sativum* (L.) was used as a test plant. Uncontaminated soil with no treatment (control) was used as reference soil to assess the impact of urban sediments and various biological remediation strategies on soil phytotoxicity. The sample was classified as toxic when the root length inhibition exceeded 20% [36].

2.4. Plant Analyses

2.4.1. Determination of Protein Content

The *T. patula* L. and *F. arundinacea* Schreb. leaf extracts were prepared using 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 M NaCl, 1 mM EDTA and 1 mM $C_6H_7NaO_6$. Obtained extracts underwent filtration on Miracloth filters and the obtained filtrates were centrifuged (15,000 g × 15 min). The supernatant was used for protein content determinations according to Bradford method [37]. The measurements were performed using a Helios Gamma spectrophotometer (Thermo Spectronic, Cambridge, UK) based on standard curves with Bovine Serum Albumin. The content of protein was depicted in mg g^{-1} of fresh mass (FM)

2.4.2. Determination of Chlorophyll Content

The *T. patula* L. and *F. arundinacea* Schreb. leaves were homogenized in an ice-cold mortar using sodium phosphate buffer, as it was described in point 2.4.1. The obtained homogenate was filtered and analyzed for chlorophyll content according to Porra et al. [38], using a Helios Gamma spectrophotometer (Thermo Spectronic, Cambridge, UK). The chlorophyll content was shown as mg g^{-1} FM.

2.5. Statistical Analysis

The two-way ANOVA was performed to test the effect of both inoculation and plants on the soil phytotoxicity, and the effect of sediment and inoculation on plant parameters (biomass, protein content, chlorophyll content, chlorophyll a/b ratio). No statistical analyses were conducted for PCDDs/PCDFs as no replicates are available. For the plant analyses statistical significance was tested separately for each plant (*T. patula* and *F. arundinacea*). The post-hoc Duncan test was used to confirm the statistically significant differences. All analyses were performed using STATISTICA 13 software.

3. Results and Discussion

3.1. The Physico-Chemical Properties and PCDD/PCDF Concentrations in Soil, Urban Sediments and Sediment-Amendment Soil

The soil used in the pot study had a loamy sand texture and the soil pH was 6.5. The soil OC content was 11.0 g kg^{-1}, which is lower than the average soil OC content in the climate zone including Poland (sub-oceanic to sub-continental) in the European LUCAS program, which was found to be 15 g kg^{-1} [39]. The concentration of PCDDs/PCDFs in soil was low, amounting to 24.8 ng kg^{-1} and 0.3 ng TEQ kg^{-1}. Similarly, concentrations of potentially toxic trace metals (PTTM) were low and ranged from 0.05 mg kg^{-1} for Cd to 28.5 mg kg^{-1} for Ba (Table 1).

Table 1. The physico-chemical characteristics of the collected urban sediment, the uncontaminated soil used in the experiment and the soil mixed in a proportion of 1:10 with the urban sediments.

Compound	Urban Sediment	Uncontaminated Soil	Urban Sediment Amended Soil
Soil pH	7.15	6.65	7.21
OC (g kg^{-1})	108	11.0	19.9
Sum of 17 PCDDs/PCDFs (ng kg^{-1})	2170	24.8	236
TEQ PCDDs/PCDFs (ng TEQ kg^{-1})	8.8	0.3	2.1
Mg (mg kg^{-1})	7040	403	1030
Ca (mg kg^{-1})	42,300	1020	5440
Fe (mg kg^{-1})	32,000	3170	6140
Zn (mg kg^{-1})	821	16.1	111
Cr (mg kg^{-1})	62.2	5.7	10.7
Cd (mg kg^{-1})	1.2	0.05	0.19
Ba (mg kg^{-1})	282	28.5	51.2
Pb (mg kg^{-1})	90.1	6.5	14.9
Cu (mg kg^{-1})	117	2.7	15.2

The fresh bottom sediments contained 35% of DM, and OC concentration was 108 g kg^{-1} DM. The sediment pH was 7.15. The sediments contained 0.70% magnesium (Mg), 0.52% potassium (K), 4.23% calcium (Ca), 3.20% iron (Fe) and 3.71% aluminum (Al). Among trace elements, only zinc (Zn) concentration exceeded the corresponding Probable Effect Concentration (PEC) value: 821 vs. 459 mg kg^{-1} [40]. Elevated concentrations were recorded for barium (Ba) and copper (Cu), 282 and 117 mg kg^{-1}, respectively, but these values did not exceed threshold values. However, PCDD/PCDF levels were high, exceeding the 0.85 ng TEQ kg^{-1} limit specified in the Sediment Quality Guideline (SQG) (http://ceqg-rcqe.ccme.ca/download/en/245) by more than 10-fold (Table 1).

The application of sediments to soil shifted its pH from neutral (pH 6.65) to slightly alkaline (pH 7.21). Also, OC increased to 19.9 g kg^{-1} after the application of sediment. The concentrations of trace elements also increased; however, they remained below the Probable Effect Concentration (PEC). Only the TEQ concentration of PCDDs/PCDFs grew significantly, exceeding the allowable limit of 0.85 ng TEQ kg^{-1} (SQG) by 2.5-fold as an effect of sediment application (Table 1).

3.2. The Effects of Urban Sediment Amendment and Applied Remediation Strategies on Soil Phytotoxicity and PCDD/PCDF Concentrations

The structure of bottom sediments renders them a perfect geosorbent for the mixture of pollutants introduced to the water environment. Consequently, the assessment of their toxicity based on monitoring of hazardous substances, such as heavy metals and PCDDs/PCDFs, does not encompass all the chemical compounds potentially present therein, nor their interactions. In this situation, the bioindication method (biotests) is a more accurate approach to assessing the toxicity of dredged sediments and may provide more useful information about the phytotoxicity and influence on soil [41–43].

In our bioassay, the application of urban sediments to soil was found to have a positive influence on plant growth, stimulating an 89% increase in *L. sativum* root length, in comparison to control soil. This increased growth can be attributed to the high nutrient content and greater Ca and Fe levels, which are known to be capable of immobilizing pollutants, especially trace elements (Table 1). This positive influence of urban sediments fell to 29% after 10 weeks of the experiment (natural attenuation strategy). However, samples inoculated with rhizobacterial strains were characterized by a better growth of *L. sativum* in comparison to the non-augmented samples, showing an increase of root growth of 38% (p87) and 65% (RP92) in comparison to control samples (Figure 2).The application of inoculation-assisted phytoremediation using both rhizobacterial inoculants and both plants further improves soil quality, with a 97% increase in *L. sativum* root growth compared to untreated samples observed in the case of simultaneous application of *F. arundinacea* and bacterial strain p87. The two-way ANOVA did not confirm, however, the influence of the bacterial inoculation and plants on the soil phytotoxicity.

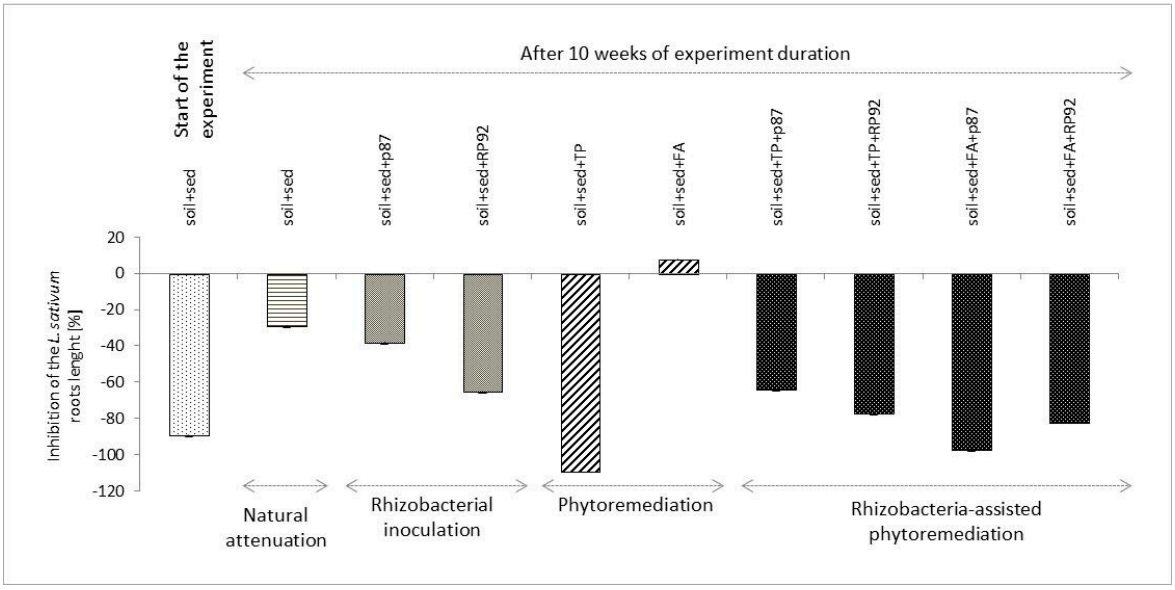

Figure 2. The effect of applied bioremediation strategies on soil phytotoxicity (negative values indicate stimulation of plant root growth).

The application of urban sediment led to nine-fold and seven-fold increases in the concentrations of the sum of 17 PCDDs/PCDFs congeners and TEQ in soil, respectively, in comparison to control soil (Table 1). The application of natural attenuation for 10 weeks increased the total PCDD/PCDF concentration by 47% (Figure 3A); however, TEQ concentration decreased by 14% in comparison to the initial value (Figure 3B). A similar situation was observed when the rhizobacterial strains were applied: Total PCDD/PCDF concentrations increased by 14% and 18% when p87 and RP92 strains were applied (Figure 3A), while TEQ decreased by 23% and 20%, respectively (Figure 3B). The phytoremediation strategy was associated with a 24% (for *T. patula*) and 10% (for *F. arundinacea*) increase of total PCDD/PCDF level (Figure 2A), as well as a 23% (for *T. patula*) and 21% (for *F. arundinacea*) decrease in TEQ value (Figure 3B).

The increasing total concentrations of PCDDs/PCDFs and the lowering of the TEQ values are related to the ongoing degradation processes. PCDDs/PCDFs are subject to both anaerobic and aerobic metabolism. Under anaerobic conditions, dechlorination of higher chlorinated congeners (mostly hexa-, hepta- and octa-chlorinated ones) of lower toxicity, reflected as low TEFs, occurs; while under aerobic conditions, lower chlorinated PCDDs/PCDFs, characterized by higher TEFs contributing in a higher extent to the TEQ, can be removed. Consequently, aerobic bacterial transformation led to the decrease in the content of lower chlorinated and thus more toxic congeners, thus leading to the reduction of the total TEQs in the studied samples.

The most effective approach, however, was the rhizobacteria-assisted phytoremediation strategy based on *F. arundinacea*. This strategy diminished the total concentration by 18% (when used with p87) or 8% (RP92) (Figure 3A), and PCDD/PCDF TEQ value by 44% (p87) or 36% (RP92) (Figure 3B). Literature data also confirms the value of *F. arundinacea* for remediation purposes. Sun et al. [44] reported the degradation of polyaromatic hydrocarbons (PAHs) in soil treated with *F. arundinacea*. The authors demonstrated that 25%, 10% and 30% of 3-ring, 4-ring and 5(+6)-ring PAHs, respectively, were removed by *F. arundinacea*, while this value was 0.6% in unplanted soil. The application of *F. arundinacea* increased the soil PAH-degrading bacterial counts and microbial activity, suggesting that the plant can restore the microbiological functioning of PAH-contaminated soil. However, it must be stated that an essential step toward the biodegradation of a given compound is the expression of the respective degradative genes in bacteria. With this in mind, Siciliano et al. [45] reported greater induction of catabolic genes involved in naphthalene degradation in the rhizosphere soil of *F. arundinacea* than

in unplanted soil. This clearly demonstrates the suitability of *F. arundinacea* in the remediation of organic compounds.

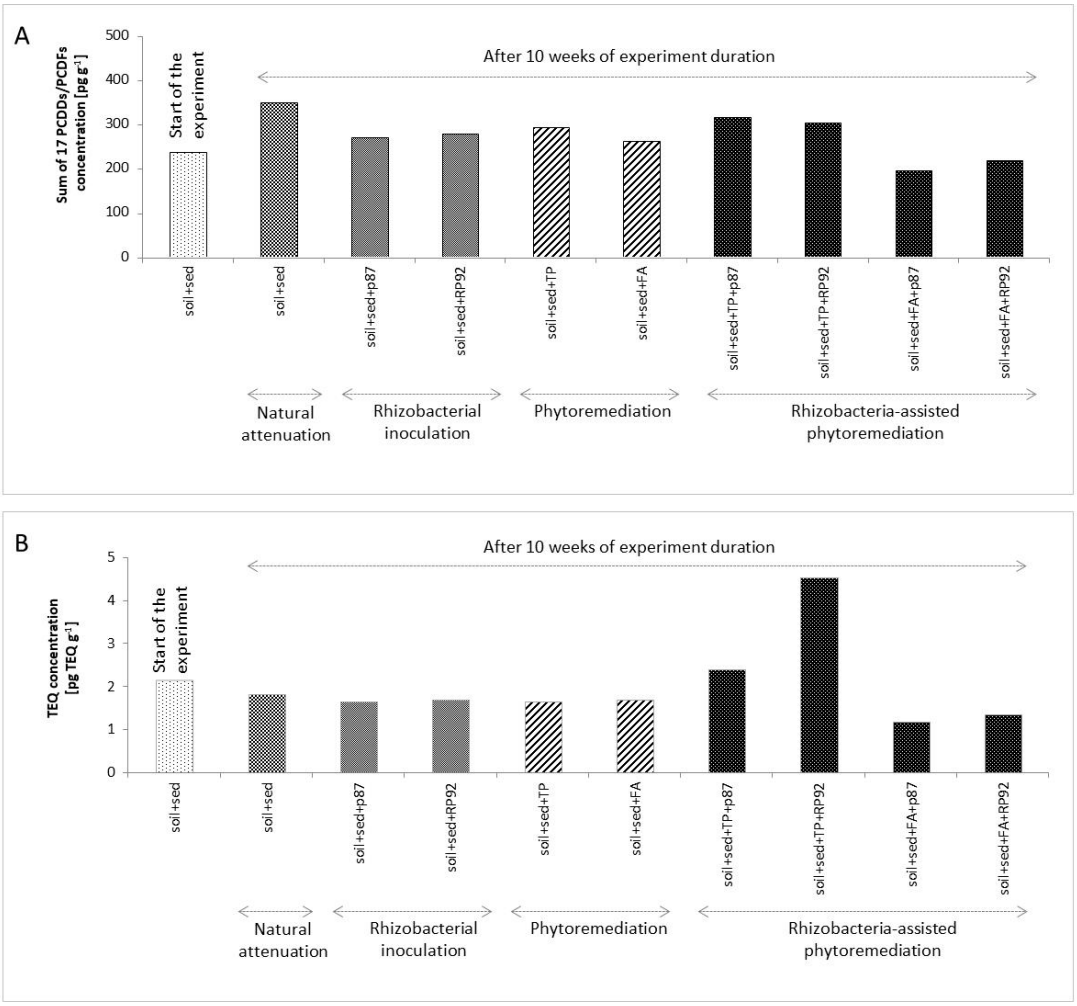

Figure 3. The effect of applied bioremediation strategies on changes in total (**A**) and TEQ (**B**) concentration of PCDDs/PCDFs.

The opposite was observed for the application of *T. patula*: Total values grew by 34% (p87) or 28% (RP92), and PCDD/PCDF TEQ values grew by 13% (p87) or 113% (RP92) (Figure 3A,B). Such increases in the total and TEQ values can be related to the transformation processes, which sometimes led to the production of intermediate compounds of higher toxicity than the parent congeners. In this case, the application of rhizobacteria-assisted phytoremediation strategy with *T. patula*, led to production of penta-chlorinated congeners (data not shown) characterized by higher toxicity in comparison to the hexa-, hepta- and octa-chlorinated compounds being the substrate for dechlorinating processes. The visual inspection of the soil-sediment samples planted with *T. patula* showed possible anoxic conditions therein (compacted, impermeable soil), facilitating dechlorination of higher chlorinated congeners and production of lower chlorinated ones of higher TEFs.

The effectiveness of rhizosphere biodegradation depends on the ability of microorganisms to adapt to a given pollutant concentration and their ability to colonize roots [46]. Kuiper et al. [47] demonstrated that naturally occurring rhizosphere biodegradation may be enhanced by the addition of microorganisms to the rhizosphere. Concluding this, in our case, both rhizobacterial inoculation and phytoremediation strategies gave similar outcomes, resulting in around a 20%–23% decline in the TEQ value. The similar effects of these strategies may be related to the fact that both led to an increase in the

activity of soil microbiota: Rhizobacterial inoculation through the artificial addition of selected strains capable of degrading the given pollutant, and phytoremediation/rhizoremediation through the existing interactions between plant exudates, soil and microorganisms. The most promising solution seems to be the rhizobacteria-assisted phytoremediation strategy; however, among the used ornamentals, only *F. arundinacea* demonstrated the capacity to reduce both PCDD/PCDF total and TEQ levels. *T. patula*, in turn, despite its positive influence on PCDD/PCDF reduction, when used alone as part of the phytoremediation strategy, increased the total and TEQ concentration when used in combination with the bacterial inoculation.

3.3. The Effects of Urban Sediment Amendments and Applied Remediation Strategies on the Biomass and Physiological Parameters of T. patula L. and F. arundinacea

Pollutant concentration in the soil is certainly a key factor determining plant tolerance or sensitivity. Nevertheless, other factors such as metal speciation, the composition of heterogeneous hydrocarbon fractions, soil-pollutant and pollutant-pollutant interactions, also have to be considered. Another aspect is the protective character of the rhizosphere microbiota, which plays an intrinsic role in the protection of plants against pathogens and stress caused by excessive concentrations of pollutants and eases the uptake of biogenic substances by a given plant [46,48,49].

From the perspective of the proper organization and management of the city space, it is important to select the most resistant plant species which both embellish the environment and resolve the pollution problem in the urban area. Therefore, the inoculation of existing soil microbiota with plant growth-promoting rhizobacteria, may not only improve plant growth, but also enhance phytoremediation rates by assisting in resource acquisition and modulating plant hormone levels, and/or by decreasing the inhibitory effects of pathogens [15,50,51].

In our case, the cultivation of *T. patula* and *F. arundinacea* following the application of urban sediments to soil led to different plant responses. *T. patula* demonstrated 67% lower biomass when grown in soil amended with urban sediments (Figure 4(A1,A2)). The inoculation of soil with bacterial strains alleviated the toxic effect caused by sediment application by 47% for p87 and 54% for RP92, in comparison to plants grown in non-inoculated soil. The addition of p87 and RP92 strains to soil following sediments application led to 2.4-fold and 2.6-fold higher production of plant biomass in comparison to non-inoculated samples (case of *T. patula*). At the same time, the inoculation did not influence the biomass of plants grown in uncontaminated soil (Figure 4(A1,A2)).

The obtained results demonstrated that soil inoculation had a positive influence on *T. patula* growth. Two-way ANOVA analysis showed that both the sediment admixture and inoculation have an influence on plant biomass. Also, Duncan post-hoc test confirmed the significantly higher biomass of plants grown in soil with sediments and inoculation, however, no differences were found between used inoculants (p87 vs. RP92). Samples without sediment demonstrated no statistically significant changes (Figure 4(A1)). Although it has been proposed to use *T. patula* as phytoremediation tool for dyes, tannery solid waste [52], soil co-contaminated by benzo[a]pyrene and metals [20], there is a considerable lack of information regarding the impact of bacterial inoculation on its growth and morphology. However, Agnello et al. [15] reported that bioaugmentation has a positive influence on plant biomass: they noted that bioaugmentation with *P. aeruginosa* had a positive effect on alfalfa biomass production, resulting in an increase of shoot biomass by as much as 56% and of root biomass by 105%.

In the case of *F. arundinacea*, the plant grown in amended soil showed only 6% lower biomass than the plants grown in uncontaminated soil. The inoculation with p87 strain did not give any positive or negative response, while inoculation with RP92 strain led to 6% higher plant biomass in comparison to plants growing on untreated soil. In contrast to *T. patula*, *F. arundinacea* demonstrated slight decreases in biomass when grown in inoculated unpolluted soil (3% for p87 and 11% for RP92), but no such changes were observed for plants grown in sediment-amended soil (Figure 4(A2)). However, the obtained results were not statistically significant (two-way ANOVA).

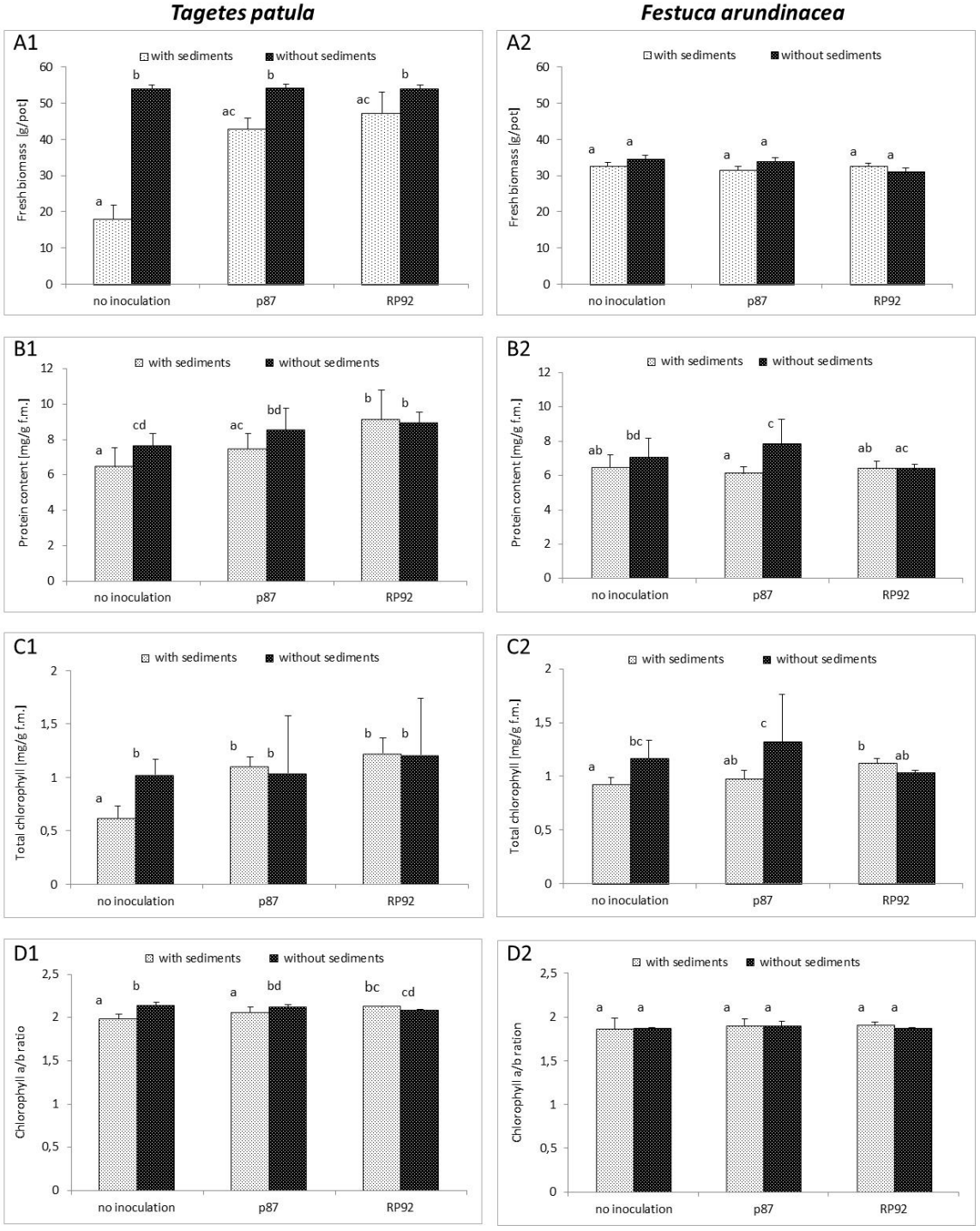

Figure 4. The effect of applied sediments and inoculation of growing medium (soil) with two bacterial strains p87 and RP92 on fresh biomass (**A**1,**A**2), protein content (**B**1,**B**2), chlorophyll content (**C**1,**C**2) and chlorophyll a/b ratio (**D**1,**D**2) in the leaf tissues of *T. patula* and *F. arundinacea* (at $p < 0.05$, the Duncan post-hoc test, the same letters indicate no statistically significant differences).

While morphological observation of the plant did not reveal the toxic effects related to the use of sediment or bacterial strains, a more diversified response was found when analyzing physiological parameters such as protein content, due to the greater sensitivity and higher degree of response to applied remediation techniques, and the range of compounds present in the sediments.

T. patula grown in sediment-amended soil showed an increase in soluble protein content. Two-way ANOVA confirmed the influence of both sediment amendment and bacterial inoculation on the protein content. The results showed that inoculation with bacterial strains led to a significant increase (Duncan post-hoc test) in protein content being 115% (for p87) and 141% (for RP82) of control values (Figure 4(B1)). In the non-inoculated soil samples, the protein content of *T. patula* was 15% lower in variants fertilized with sediment than in the unfertilized ones. The obtained differences were statistically significant (Duncan post-hoc test).

In contrast, in the case of *F. arundinacea*, sediments had a significant influence on protein content (two-way ANOVA), while inoculation did not affect it. Duncan post-hoc test, however, revealed the statistically significant difference in variant inoculated with p87 and no sediment admixture. In this case, application of p87 strain to the unamended soil led to a 12% increase in the protein content, while simultaneous application of both p87 and sediment resulted in lowering the protein content (Figure 4(B2)).

From the physiological point of view, it is known that soluble protein content decreases as plant senescence-related processes continue [53,54]. This is related to the initiation of the N-remobilization process, during which proteinase activity increases, leading to the degradation of protein to amino acids. The resulting amino acids and/or peptides are then transported through the phloem sap to the growing organs [55]. With this in mind, the increase in soluble protein content observed in *T. patula* tissues grown in inoculated soil may indicate a delay of senescence processes. This increase in soluble protein content is particularly noticeable after sediment application. Considering the toxicity of the sediment used in the experiment, the above described relationship can also indicate that the introduced rhizobacterial strains have a protective effect on studied plants that is reflected in the prevention of plant premature senescence. RP92 proved to have a stronger protective effect in this case.

In the case of total chlorophyll content of *T. patula*, two-way ANOVA demonstrated that sediments have no influence, unlike the type of inoculation that was found to significantly affect the content of chlorophyll. Duncan post-hoc test demonstrated, in turn, that the use of urban sediment significantly lowered the total chlorophyll content in green tissues of *T. patula* grown in non-inoculated variant, being only 60% of that of the value measured in plants grown in non-amended soil (Figure 4(C1)). In this case, the inoculation of sediment-amended soil with p87 and RP92 significantly increased the chlorophyll content to 178% and 198% of non-inoculated and unamended control, respectively (Duncan post-hoc test). Moreover, RP92 increased the chlorophyll content even in non-fertilized plants to 118% of non-amended and non-inoculated control values; however, this increase was not statistically significant (Figure 4(C1)). Regarding the chlorophyll a/b ratio, the two-way ANOVA confirmed the influence of both the sediment admixture and inoculation on the obtained results. Duncan post-hoc test revealed significantly higher chlorophyll a/b ratio in plants grown in soil amended with sediment and inoculated with RP92, in comparison to non-inoculated samples as well as ones inoculated with p87 (both amended with sediments) (Figure 4(C1)). The unamended samples inoculated with RP92 demonstrated a significantly lower chlorophyll a/b ratio, when compared to the unamended and non-inoculated sample (Figure 4(C1)).

For *F. arundinacea*, two-way ANOVA demonstrated a contrary effect to *T. patula*: In this case, the chlorophyll content was dependent on the sediment application, while no such effect was observed for inoculation. Duncan post-hoc test revealed the significant increase in the chlorophyll content in sediment amended samples, in comparison to unamended ones. Moreover, significantly higher chlorophyll content was observed in plants grown in soil amended with sediments and inoculated with RP92, in comparison to the non-inoculated variant (Figure 4(C2)). Inoculation with p87, in turn, led to significantly higher chlorophyll content, but only when compared with samples without sediments and without inoculation. In the case of chlorophyll a/b ratio, the statistical analyses showed neither the influence of sediments application, nor the type of inoculation (Figure 4(D1,D2)).

Our findings indicate that the soil amended with the urban sediment caused a significant reduction of chlorophyll content in both *T. patula* and *F. arundinacea;* while soil inoculation with bacterial strains

not only suppressed this effect, but also led to an increase in chlorophyll content in comparison to control samples. The decrease of the chlorophyll content observed in investigated plants due to the application of sediments may be related to the presence of xenobiotics in the sediment. The decrease in chlorophyll content in leaves is one of the first symptoms of plant senescence, during which the breakdown of the thylakoid membranes and degradation of thylakoid-bound proteins occur. Our findings suggest that the use of sediments can exacerbate this process, while the application of bacterial strains protects plants from premature senescence. Furthermore, the reduced chlorophyll a/b ratio may indicate the progress of the aging process of *T. patula* and *F. arundinacea* grown in soil treated with sediments without simultaneous inoculation. Similarly, Nath et al. [56] demonstrated a linear decrease of chlorophyll a/b ratio in *Arabidopsis thaliana* during natural leaf senescence, along with a decrease in total chlorophyll content. The authors noted that with gradual senescence, differential degradation of chlorophyll a and chlorophyll b leads to changes in chlorophyll a/b ratio.

In conclusion, *T. patula* is more susceptible to the range of xenobiotics in the tested sediments, but the application of bacterial strains alleviated the phytotoxicity, especially the RP92 strain. The use of bacteria also gives positive results when the soil is not amended with sediment. In the case of *F. arundinacea*, the use of both sediment application and inoculation with rhizobacterial strains promotes a good plant physiological status; while in sediment-free soil, only strain p87 seems to be beneficial.

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