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Impact of rhizobacterial inoculants on plant growth and enzyme activities in soil treated with contaminated bottom sediments

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ABSTRACT

The impact of contaminated bottom sediments on plant growth and soil enzyme activities was evaluated in a greenhouse pot study. The sediments were moderately contaminated with zinc and heavily contaminated with polycyclic aromatic hydrocarbons and polychlorinated dibenzo-*p*-dioxins and furans. The sediments were mixed with soil and planted with either *Festuca arundinacea* or *Tagetes patula*. The capacity of two rhizobacterial strains (*Massilia niastensis* P87 and *Streptomyces costaricanus* RP92), previously isolated from contaminated soils, to improve plant growth under the chemical stress was tested. Application of sediments to soil was severely phytotoxic to *T. patula* and mildly to *F. arundinacea*. On the other hand, the addition of sediments enhanced the soil enzymatic activity. Inoculation with both bacterial strains significantly increased shoot (up to 2.4-fold) and root (up to 3.4-fold) biomass of *T. patula*. The study revealed that the selected plant growth-promoting bacterial strains were able to alleviate phytotoxicity of bottom sediments to *T. patula* resulting from the complex character of the contamination.

KEYWORDS

Bottom sediment; plant growth promoting bacteria; rhizosphere

Introduction

Contamination of bottom sediments is a widespread problem of significant environmental concern due to the risks of contaminant mobilization under certain conditions and their potentially harmful effects on the environment and human health. Large volumes of contaminated sediments can be produced, for example, within Europe alone estimations of sediments production are around 100 to 200 million cubic meters per year, and these need to be sustainably managed (Akcil et al. 2015). Contaminants, including trace metals and persistent organic pollutants (POPs) (e.g. polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated furans (PCDFs), are transported within surface waters and accumulate in the bottom sediments of rivers and natural or artificial lakes (Urbaniak et al. 2010). Bottom sediments can also be accidentally incorporated into soils, for example, during intense flooding events. Many of these contaminants are characterized by a high persistence and toxicity, low solubility and, in the case of organic pollutants, slow degradation rate (Macek et al. 2004). According to a national monitoring of sediments carried out in Poland in 2012, only 20% of lake sediments, 43% of river sediments

and 58% of channel and artificial reservoir sediments were not contaminated with trace elements (TE). In general, lake sediments were more frequently contaminated with PAHs and OCPs than river sediments (Chief Inspectorate of Environmental Protection 2017).

Phytoremediation uses plants and their associated microorganisms to remove, stabilize or detoxify pollutants in contaminated substrates. Recently, phytoremediation was proposed as a sustainable management strategy for contaminated dredged sediments (Doni et al. 2015). The application of these plant-based technologies in dredged sediments is still an emerging technology and few studies have evaluated its effectiveness in this type of contaminated substrate. Doni et al. (2015) evaluated the combined use of compost addition and different plant species, including *Paspalum vaginatum* Sw., *Spartium junceum* L., and *Tamarix gallica* L., to remove trace metals (Cd, Ni, Zn, Pb, and Cu) and total petroleum hydrocarbons from contaminated sediments.

Moreover, plant-associated bacteria have been shown to improve phytoremediation techniques by increasing plant growth and tolerance and/or modifying contaminant availability (Weyens et al. 2009; Sessitsch et al. 2013; Kidd et al. 2017). Plant growth promoting rhizobacteria can enhance growth through the production of phytohormones [such as

indoleacetic acid (IAA)], suppression of stress ethylene production (due to ACC deaminase activity), or the release of essential nutrients (e.g. due to the presence of N₂-fixers, phosphate-solubilizers, and siderophore-producers), or the induction of plant defense mechanisms (Weyens et al. 2009). They can also modify contaminant bioavailability through their biological transformation (e.g. accelerated degradation of organic contaminants, chelation and/or precipitation of trace metals) (Sessitsch et al. 2013). Most studies have focused on contaminated soils, whereas the application of bacterial inoculants in the remediation of sediment-contaminated soils has not to date been addressed, and could offer a potentially useful method for managing this type of substrate.

In addition to harmful contaminants, sediments also contain potentially useful nutrients [such as nitrogen (N) or phosphorus (P)] and organic matter which can aid plant growth or enrich the soil with carbon (C). Thus, these types of sediments can potentially be used as part of reclamation purposes (in wastelands, landfills) or in urban green areas (lawns, flowerbeds). However, the consequences of sediment application to soil must be carefully assessed including their impact on plant growth, soil microbial activity and contaminant bioavailability.

The objectives of this study were to evaluate: (1) the impact of the application of contaminated bottom sediments to soil on plant growth and soil enzyme activities, and (2), the effectiveness of rhizosphere inoculation with pre-selected rhizobacterial strains in alleviating contaminant toxicity to plants.

Materials and methods

Experimental design

Contaminated sediments were collected in July 2014 from a sequential sedimentation -biofiltration system located in Lodz, Poland, on the Sokolowka River. Fresh sediments (35% dry matter) were mixed with an agricultural uncontaminated soil in a 1:10 w/w proportion and transferred into 2 kg pots. Four representative samples of homogenous soil:sediment mixture were collected to record the initial content of organic pollutants and TE solubility. The uncontaminated soil was collected from Osiny/Pulawy and was sieved to 2 mm. It was a Haplic Luvisol according to FAO classification (IUSS Working Group WRB 2015), with a loamy sand texture and soil pH of 6.7 and soil organic carbon (SOC) content of 11.0 g kg⁻¹. Soil:sediment mixtures were left for 4 weeks to allow for equilibrium. For control purposes pots were also prepared using uncontaminated soil with no addition of sediments. The filled pots were then seeded with two plant species: tall fescue — *Festuca arundinacea* Schreb. (Poaceae) (FA) and *Tagetes patula* L. (Asteraceae) (TP). *F. arundinacea* was seeded at a rate of 2 g pot⁻¹, while *T. patula* was seeded at a rate of 15 seeds pot⁻¹; after germination they were reduced to 10 plants. *F. arundinacea* has been tested in bioremediation of organic contaminants and phytostabilization of metal polluted soils and mine tailings (Pierzynski et al. 2002; Ho and Banks, 2007). *T. patula* is a

low-growing ornamental plant that is very often used in urban green areas and gardens. Therefore resistance of this plant to soil and air contamination is important for the effectiveness of green area management in cities, often utilizing public money. In parallel to planted pots, reference unplanted pots were established in order to differentiate between plant-bacterial interactions and bacterial-induced effects.

Two rhizobacterial strains were used as inoculants in this study which were previously isolated from contaminated soils (with either trace metals or organic compounds). *Massilia niastensis* P87 was isolated from the rhizosphere of *Festuca rubra* growing on mine tailings with elevated concentrations of Cd, Pb and Zn (Becerra-Castro et al. 2012). Strain P87 is an IAA-producer and Cd/Zn-resistant, and was previously found to increase the biomass of both *Salix viminalis* and *F. arundinacea* growing in Cd/Zn-enriched Hoagland solutions (Becerra-Castro et al. 2012). *Streptomyces costaricanus* RP92 was isolated from the rhizosphere of *Cytisus striatus* growing in hexachlorocyclohexane (HCH)-contaminated soil and was characterized as an IAA- and siderophore-producer (Becerra-Castro et al. 2011). Strain RP92 was previously shown to stimulate the growth of *Lupinus luteus* in diesel-contaminated soils, as well as to improve the dissipation of diesel range organics (Balseiro-Romero et al. 2016).

Fresh cultures of bacterial strains were grown in 869 liquid medium (Mergeay et al. 1985) for 24 h. Five millilitres of this pre-culture were then transferred into fresh 869 liquid medium and grown for 12 h. After this, bacterial biomass was harvested by centrifugation (6000 rpm, 15 min), washed once with sterile 10 mM MgSO₄, and re-suspended in 10 mM MgSO₄ to an OD₆₆₀ of 1.0 (about 10⁷ cells per mL). Each plant pot was inoculated when plants were germinating (4 weeks after seeding) with 100 mL of each bacterial suspension. The same amount of sterile 10 mM MgSO₄ was added to non-inoculated pots. The inoculation was repeated after 3 weeks using the same procedure.

The pot study was run for 10 weeks in a greenhouse under controlled conditions (supplemental light and 27/20 °C day/night temperatures) with three replicates for each combination of the plant species and inoculant and the soil treatment. The pots were watered with deionized water as needed. The full experimental scheme is presented in Table 1.

After 10 weeks of growth the plants were harvested, dried and weighed to determine the dry shoot weight (DW) yield. After harvesting, soils from each treatment were mixed, sieved to 2 mm to remove plant residues, and aliquots of soil were collected for further analyses. Each soil sample was divided into two portions: fresh soil samples were used for the analysis of soil enzymes whereas air dried samples were used to determine total and extractable TE concentrations and soil pH.

Analysis of sediment and soils

Soil pH was measured in H₂O using a 1:2.5 soil:solution ratio. Total organic carbon (OC) and nitrogen (N) were

analyzed by combustion with a CN analyzer (Elementar, vario Macro cube). Soil and sediment samples were digested in a 3:1 mixture of concentrated HNO₃:HCl in teflon PFA vessels in a microwave accelerated reaction system (MarsXpress; CEM Corp., USA) and total concentrations of metals were analyzed by ICP-MS (Agilent 7500ce). Metal availability was assessed after extracting soil samples with 1 M NH₄NO₃ in a soil:solution ratio of 1:2.5 (w/v) for 2 h. Concentrations of Cd, Pb, and Zn were analyzed by ICP-MS in the filtered soil extracts.

Sixteen PAH compounds: and three DDT isomers (p,p'DDE, p,p'DDD, p,p'DDT) were determined by GC-MS technique in SIM (Selected Ion Monitoring) mode after extraction of soil samples in ASE200 and clean-up in micro-columns filled with silica gel (PAHs) or neutral alumina (DDTs). The concentration of PAHs was expressed as sum of 16 compounds (to address impact of the whole range of PAHs) or as sum of 9 compounds (naphthalene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene) in order to evaluate the sediments with respect to the PEC (*Probable Effect Concentration*) criteria (MacDonald

et al. 2000). PEC is the concentration above which a toxic effect of a contaminant is likely to be observed.

The analysis of the 17 toxic congeners of PCDD/PCDF was performed according to PN-EN 1948-3 (2002) and US EPA Method 1613 (1994) using the isotope dilution method and 6890 N High Resolution Gas Chromatography/High Resolution Mass Spectrometry system (Agilent Technologies) with a DB-5MS column. The final results were expressed as the Toxic Equivalency (TEQ) of each sample, operationally defined by the sum of the concentrations of each congener in the mixture multiplied by its Toxic Equivalency Factor (TEF) (Van den Berg et al. 2006).

Soil enzyme activities were determined at harvest in the different treatments. Acid and alkaline phosphomonoesterase activity were determined at pH 6.5 and 11.0, respectively, with p-nitrophenyl phosphate as a substrate, following the method of Tabatabai and Bremner (1969). The activity was expressed as µg produced p-nitrophenol (PNP) per g per h. Dehydrogenase activity was determined as described by Casida et al. (1964), and the activity was expressed as µg TPF g⁻¹ h⁻¹.

Results and discussion

Quality of the bottom sediments

The bottom sediments contained 0.70% magnesium (Mg), 0.52% potassium (K), 4.23% calcium (Ca), 3.20% iron (Fe), and 3.71% aluminum (Al). Sediment pH was 7.15 and it contained 108 g OC and 8.3 g total N kg⁻¹. The sediments presented elevated concentrations of TE compared to their geochemical background levels as established by Bojakowska and Sokołowska (1998) (Table 2). However, only the Zn concentration exceeded the corresponding PEC value (MacDonald et al. 2000). It is worth noting that TE concentrations in the bottom sediments were substantially lower than the threshold values set by the EU for sewage sludge application in agriculture (CEC 1986) (Table 2). The

Table 1. Experimental scheme of the pot experiment.

Sediment rate (%)	Plant species	Inoculation	Abbreviations
10	<i>F. arundinacea</i>	P87	S-FA-P87
10	<i>F. arundinacea</i>	RP92	S-FA-RP92
10	<i>F. arundinacea</i>	No inoculation	S-FA-0
10	<i>T. patula</i>	P87	S-TP-P87
10	<i>T. patula</i>	RP92	S-TP-RP92
10	<i>T. patula</i>	No inoculation	S-TP-0
10	Unplanted	P87	S-UNP-P87
10	Unplanted	RP92	S-UNP-RP92
10	Unplanted	No inoculation	S-UNP-0
0	<i>F. arundinacea</i>	P87	0-FA-P87
0	<i>F. arundinacea</i>	RP92	0-FA-RP92
0	<i>F. arundinacea</i>	No inoculation	0-FA-0
0	<i>T. patula</i>	P87	0-TP-P87
0	<i>T. patula</i>	RP92	0-TP-RP92
0	<i>T. patula</i>	No inoculation	0-TP-0
0	Unplanted	No inoculation	0-UNP-0

Table 2. Total concentrations of trace elements, PAHs and PCDD/PCDF in the bottom sediment of Sokolowka River (TE and PAHs in mg kg⁻¹, PCDD/PCDF in ng kg⁻¹).

Contaminant	Mean content	Geochemical background ^a	PEC ^b	Threshold for soil ^c	Threshold for sewage sludge ^d
Antimony (Sb)	8.7	–	–	–	–
Arsenic (As)	8.2	5	33	20	–
Barium (Ba)	282	50	–	400	–
Bismuth (Bi)	0.7	–	–	–	–
Cadmium (Cd)	1.2	0.5	4.98	3	20
Cesium (Cs)	42.9	–	–	–	–
Chromium (Cr)	62.2	6	111	300	500
Copper (Cu)	117	7	149	150	1000
Gadolinium (Gd)	4.2	–	–	–	–
Lanthanum (La)	22.2	–	–	–	–
Lead (Pb)	90	15	128	250	750
Nickel (Ni)	39.6	5	48.6	150	300
Thalium (Tl)	0.3	–	–	–	–
Tin (Sn)	9.3	–	–	20	–
Vanadium (V)	57.4	–	–	–	–
Zinc (Zn)	821	73	459	500	2500
9 PAHs sum	30	–	22.8	–	–
PCDD/PCDF sum	2173	–	–	–	–

^aBojakowska and Sokołowska (1998).

^bMacDonald et al. (2000).

^cMoE (2016).

^dCEC (1986).

Table 3. Impact of the soil inoculation and the plant species on soil pH and trace element extractability in 1 M NH₄NO₃ in the soil amended with the sediment at harvest.

Element	Plant species	Inoculant			Initial value after sediment addition	Reference ^A
		No inoculation	P87	RP92		
pH	FA	7.48a†‡	7.35a‡	7.28a†	7.21	6.68
	TP	7.35a‡	7.53a†	7.48a†		
	Unplanted	7.50a†	7.43a†‡	7.50a†		
Ba (mg kg ⁻¹)	FA	3.92 a ^B ,C†	3.93a†	3.82a†	3.90	0.62
	TP	3.88a†	3.67a†	3.81a†		
	Unplanted	4.19a†	3.82a†	4.10a†		
Zn (µg kg ⁻¹)	FA	80.9a†	70.7a†	63.5a†	84.6	11.4
	TP	66.7a†	74.1a†	61.4a†		
	Unplanted	124.5a‡	96.4a‡	116.2a‡		
Cu (µg kg ⁻¹)	FA	139.3a†	149.4a†	132.4a†	138.0	52.9
	TP	127.4a†	152.6a†	141.8a†		
	Unplanted	135.4a†	136.0a†	146.9a†		
Sb (µg kg ⁻¹)	FA	35.2a†	31.8a†	32.8a†	34.3	0.7
	TP	34.8a†	35.1a†	35.6a†		
	Unplanted	42.7a†	39.5a†	41.1a†		

^AUntreated soil (no sediment, no plant and no inoculation) at time of harvest.

^BMeans preceded by different symbols are significantly different between the plant species according to the Tukey's test with $p < 0.05$.

^CMeans followed by the same letters are not significantly different between the inoculation types according to the Tukey's test with $p < 0.05$.

concentration of Zn in the sediment substantially exceeded its threshold value for of soil when used in agriculture (MoE 2016). Elevated concentrations, but not exceeding threshold values, were recorded for barium and copper (Table 2).

The sediment was heavily contaminated with PAHs: the sum of 9 PAHs in the sediment exceeded the PEC value (Table 2). 4-ringed and 5-ringed hydrocarbons constituted 56% and 40% of the total amount of PAHs, respectively, with fluoranthene and benzo(b)fluorantene as the most abundant (30% and 17%, respectively). No elevated DDT concentrations were measured. All DDT isomers were under the detection limit of 0.01 mg kg⁻¹. Threshold concentrations for PCDDs/PCDFs in sediments do not exist in Poland; moreover these compounds are rather rarely measured due to the high cost of the analysis. The PCDDs/PCDFs concentrations measured for the sediment used in our study (2173 ng kg⁻¹ and 8.82 ng TEQ kg⁻¹) can be considered as high according to the Sediment Quality Guidelines (SQG) (<http://st-ts.ccme.ca/en/index.html>). According to the SQG, sediments containing amounts higher than 0.85 ng TEQ kg⁻¹ are regarded as polluted, while those exceeding the level of 21.5 ng TEQ kg⁻¹ are likely to have adverse health effects. Therefore the studied sediments exceeded the sediment pollution limit more than 10-fold indicating its high pollution level. A previous study conducted in the Sokolowka river by Urbaniak et al. (2015) showed an even higher contamination of urban reservoirs sediments with concentrations ranging from 12 to even 15,536 ng kg⁻¹, while the concentrations expressed as TEQ varied from 0.75 to 21.51 ng TEQ kg⁻¹. Overall, the sediments reflected a complex contamination consisting in PAHs, PCDDs/PCDFs and zinc, with other trace elements (Ba, Cu) further increasing risk of phytotoxic effects of sediment application to soil.

Effects of sediment addition and bacterial inoculation on soil properties

Amending the soil with sediments led to an increase in total TE concentrations. The mean increase in the different TE

was as follows: from 5.7 to 10.7, 0.05 to 0.19, 28.5 to 51.2, 6.5 to 14.9, 16.1 to 111.4, and 2.7 to 15.2 mg kg⁻¹, for Cr, Cd, Ba, Pb, Zn, and Cu, respectively. Despite this increase in TE concentrations, the values were still far from threshold concentrations considered as potentially phytotoxic, at least in stable soil systems where metals are not freshly introduced into the soil (Kabata-Pendias 2010). The NH₄NO₃-extractable TE concentrations, determined in the soil both before plant seeding and after harvesting, generally confirmed the limited mobility of these metals. The mean concentrations of selected NH₄NO₃-extractable TE in the soil-sediment mixture were low (for example 0.001, 0.001, 3.9, 0.003, 0.08, 0.14 mg kg⁻¹, for Cr, Cd, Ba, Pb, Zn, and Cu, respectively) at the beginning of the study and did not change dramatically after harvest (Table 3). Inoculating the soils did not significantly affect TE extractability (Table 3). Soil pH increased from 6.5 to above 7.2 after the sediment incorporation into the soil. Neither soil inoculation nor plant species had any influence on soil pH in the sediment treated soil (Table 3). Sediment application to soil raised SOC from 11.0 up to 19.9 g kg⁻¹.

The concentration of 16 PAHs increased from 0.15 to 3.49 mg kg⁻¹ after application of the sediment to the soil. Concentrations of 6 PAHs (naphthalene, phenanthrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(a)pyrene) of 10 listed in the national regulations exceeded thresholds for individual compounds after the sediment addition (MoE 2016). Similarly, application of the sediment substantially raised PCDDs/PCDFs concentrations from 0.32 to 2.13 ng TEQ kg⁻¹.

It is worth noting that soil inoculation with the P87 and RP92 bacterial strains did not stimulate PAHs dissipation as indicated by comparing the sum of 16 PAHs before plant seeding and after harvest (Table 4). There were also no significant differences between the two inoculants and non-inoculated soil. The only differences at harvest were observed between plant species. The sum of 16 PAHs was higher in pots where tall fescue was grown as compared to the pots

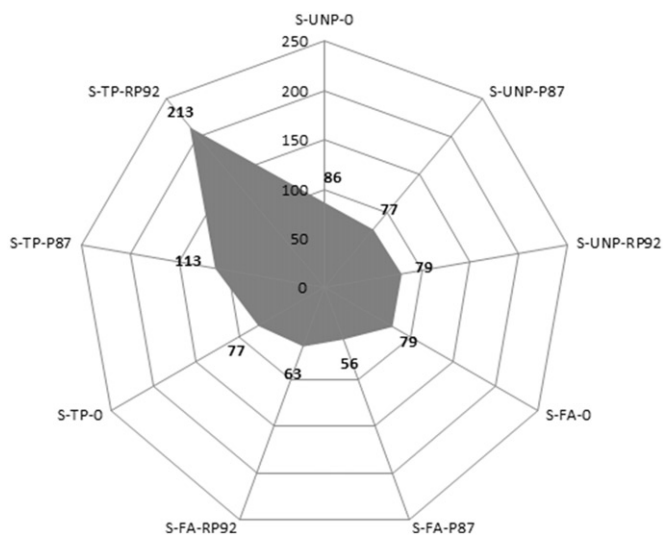
Table 4. Impact of the soil inoculation and the plant species on the content of 16 PAHs in the soil amended with the sediments at harvest.

Plant species	Inoculant			Initial content after sediment addition	Reference ^A
	No inoculation	P87	RP92		
<i>Festuca</i>	3.98 a ^B , c [‡]	3.76 a [†]	4.07 a [‡]	3.49	0.22
<i>Tagetes</i>	3.70 a [†]	3.70 a [†]	3.81 a [†]		
unplanted	3.76 a ^{†‡}	3.65 a [†]	3.78 a [†]		

^AUntreated soil – no sediment, no plant and no inoculation.

^BMeans preceded by different symbols are significantly different between plant species according to the Tukey's test with $p < 0.05$.

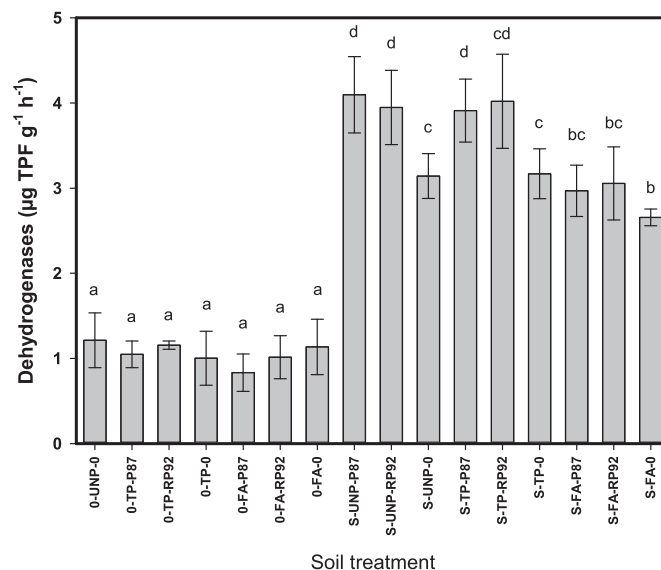
^CMeans followed by the same letters are not significantly different between the inoculation types according to the Tukey's test with $p < 0.05$.

**Figure 1.** Impact of plant species and rhizosphere inoculation on change of PCDD/PCDFs concentration (% of initial concentration).

with *Tagetes* and the unplanted soil (Table 4). This was recorded in non-inoculated and RP92-treated soils.

The PCDDs/PCDFs concentration in sediment treated soil, expressed as TEQ, in non-inoculated soils decreased after harvest, regardless of the plant species grown (77–86% of the initial value, with the lowest concentration in the *Festuca* pots and the highest in the unplanted soil) (Figure 1). A reduction in TEQ was also observed in all unplanted soils. However, P87 and RP92 bacterial strains led to a more drastic TEQ reduction than that recorded for non-inoculated soils. The combined phyto- and rhizoremediation approach (tall fescue combined with the inoculation) was the most effective in reducing the PCDDs/PCDFs concentration, as expressed by the TEQ value. FA-P87 and FA-RP92 reduced the TEQ to 56 and 63% of the initial level in the soil mixed with the sediments whereas in soils with tall fescue but without inoculation TEQ fell to 79% of the initial value (Figure 1). Non-inoculated *T. patula* reduced the PCDDs/PCDFs to 77% of the initial TEQ, which is similar to values obtained in FA-0. However, the interaction of *T. patula* and the bacterial inoculants accelerated transformations amongst congeners of the PCDDs/PCDFs. As a result, TEQ increased in S-TP-P87 and S-TP-RP92 to up to 113 and 213% of the initial concentration (Figure 1).

The addition of the bottom sediments to the soil led to enhanced activities of dehydrogenase and alkaline

**Figure 2.** Soil dehydrogenases activity (mean \pm standard deviation) in response to the bottom sediment application and rhizosphere inoculation by bacteria strains.

phosphatase enzymes. Dehydrogenase activity was increased by three- to fourfold in all the amended soils, and this was the case for both planted and unplanted soils (Figure 2). Activities in the inoculated sediment-amended soils were slightly higher than those recorded in corresponding non-inoculated soils. However, these differences were in most cases not statistically significant. The increase in the dehydrogenase activity is likely due to the effect of the exogenous organic matter introduced along with the sediment. High dehydrogenase activity in S-TP-P87 and S-TP-RP92 confirmed intensive soil microbial processes, also likely to be responsible for the observed transformations of PCDDs/PCDFs. The effect of sediment addition was even more pronounced on alkaline phosphatase activity (Figure 3). The production of PNP increased from 10 to approx. $50 \mu\text{g g DM soil}^{-1} \text{ h}^{-1}$ after the sediment application. Generally, plant growth led to a slight reduction in the phosphatase activity compared to unplanted soils, regardless of the presence, or lack of, sediments. Inoculation with either P87 or RP92 strains did not have a significant effect on the alkaline phosphatase activity in the sediment-treated soils. In contrast to what was observed for alkaline phosphatase and dehydrogenase, the addition of sediment did not have any significant effect on acidic phosphatase activity, partly due to the pH shift after sediment application.

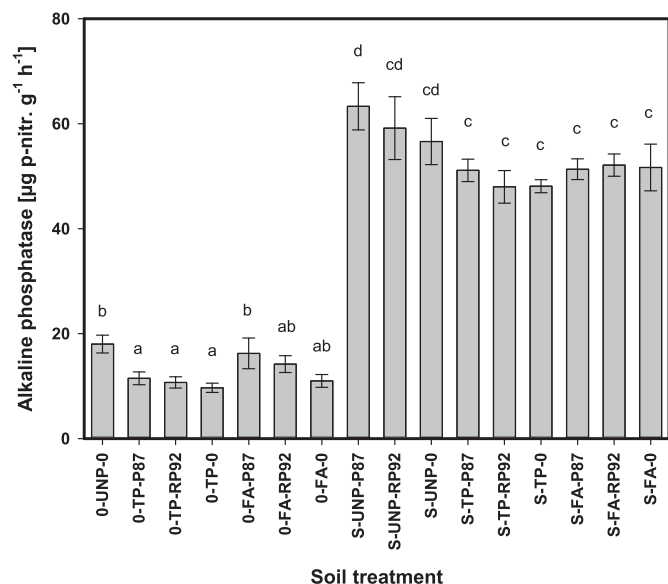


Figure 3. Alkaline phosphatase activity (mean \pm standard deviation) in response to bottom sediment application and rhizosphere inoculation by bacteria strains.

Plant performance and yield

No visible toxicity symptoms were observed in *Festuca* growing in the soil mixed with the bottom sediments. However, the yield of this species was lower in some combinations of sediment treated soil than in corresponding combinations in uncontaminated soil (Figure 4). In addition, bacterial inoculation did not significantly affect *Festuca* biomass production, and this was observed in both the soil-sediment mixture and the uncontaminated control soils (Figure 4).

In contrast, the growth of *T. patula* was severely stunted in the non-inoculated soil mixed with the bottom sediment (Figure 5). Plant leaves were chlorotic and the flowers did not fully develop. Leaves represented a significantly lower percentage of the whole plant biomass (40.3%) in those plants grown in the non-inoculated soil-sediment mixture compared to either the inoculated soil-sediment mixture (43.3–44.5%) or the uncontaminated control soil (46.3–47.6%). Apparently, there was a strong toxicity to plant roots in S-TP-0 soil, exhibited by more than eightfold lower root biomass than in the soil without the sediment addition (0.63 vs 5.0–5.3 g pot⁻¹). In this plant species, a strong effect of bacterial inoculation on plant growth was observed. Inoculation of the soil with both P87 and RP92 strains significantly improved the growth of *Tagetes* and the chlorosis were almost fully alleviated (Figure 5). Shoot DW yields of these plants were doubled after inoculation and almost reached the yields obtained in uncontaminated soils (Figure 4). Both inoculants had a similar beneficial effect on plant growth in terms of shoot DW yield (Figure 5). Inoculation had no effect on shoot DW yields of *Tagetes* grown in uncontaminated soils, suggesting that the differences observed between the non-inoculated and inoculated sediment-contaminated soils were due to a bacterial-induced reduction in toxicity of sediments or improved plant resistance to the stress. Root biomass in the sediment treated soil increased from 0.63 to 1.85 g pot⁻¹ in S-TP-P87 and 2.17 g

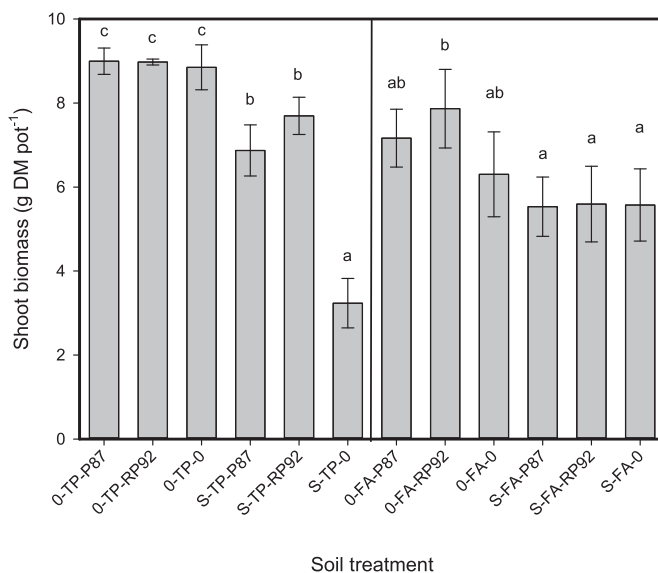


Figure 4. Effect of sediment and inoculation on *Festuca* and *Tagetes* yield (mean \pm standard deviation).

pot⁻¹ in S-TP-RP92 (2.9- and 3.4-fold increase, respectively). It is worth emphasizing that soil texture affect bacterial colonization of the rhizosphere and activity of strains introduced into soil. Afzal et al. (2011) reported much greater colonization and gene abundance and expression of two inoculated strains in loam or loamy sand than in acidic sandy soil. In our study we applied inoculants to loamy sand with neutral pH which conditions clearly favored the effective inoculation. Soil inoculation selected in our study seems to be also more effective than applying the strains by seed imbibition (Afzal et al. 2012).

Judging from the concentrations of TE and organic pollutants the observed strong toxicity to the *Tagetes* and the mild toxicity to *Festuca* was most likely due to complex interactions. None of the contaminants were present in extreme concentrations (Table 2). However their cumulative effect could have been detrimental to *Tagetes*. The change of redox conditions after sediment application to soil might have induced the release and transformations of pollutants to forms more accessible to plants.

The positive effect of the rhizobacterial inoculation on *Tagetes* performance in sediment treated soil was apparently due to an improvement in the overall growth of plants and their increased resistance to contamination. The analysis of POPs concentrations and TE extractability at the beginning of the study and harvest did not reveal any significant decreases. It must be emphasized that the observed increase in the TEQ concentration of PCDD/PCDFs after soil inoculation, especially after RP92 addition, apparently did not result in an increment in PCDD/PCDFs phytotoxicity. The inoculation of *Tagetes* rhizosphere rather stimulated transformations within their congeners. The plant biomass or yield reduction were not correlated to enzyme activities across all variants in sediment treated soils.

The apparent increase in *Tagetes* root biomass observed after P87 and RP92 application to the sediment contaminated soil indicated reduced toxicity of this contaminant mixture to plants. The production of IAA is known to



Figure 5. *Tagetes patula* performance before harvest in sediment-treated and untreated soils as dependent on the inoculation strains. Lower row: soil treated with sediment, from the left – no inoculation, RP92, P87. Upper row: uncontaminated soil, from the left – no inoculation, RP92, P87.

significantly improve plant growth and biomass production (Shilev et al. 2006; Dell'Amico et al. 2008). Both the bacterial strains used in this study were capable of producing IAA. Examples can also be found in the literature showing bacterial-induced enhanced growth of plants in soils contaminated with petroleum hydrocarbons (Khan et al. 2013). Microbial degradation of PAHs in the rhizosphere might involve several mechanisms – not only mineralization of a compound but also intracellular detoxification process (Johnsen et al. 2005). Similarly, for PCDDs/PCDFs rhizodegradation involving interactions between plant roots, plant exudates, and microorganisms is one of the most effective remediation processes (Macek et al. 2004). The effectiveness of a given rhizobacteria inoculant is plant species-specific – differences between rhizosphere or root colonization by the same strains are observed for different plant species, even within the same genus (Fatima et al. 2016).

The use of bottom sediments as a soil amendment has rarely been addressed. Bottom sediments were found to stimulate maize yield at a 5% rate of addition, while at higher doses the sediment reduced plant yield (Baran et al. 2013). These papers and our results reveal that the sediments would be better used in remediation processes than applied in forage production systems. Furthermore, the

potential sediment rates for remediation purposes should be carefully evaluated.

Conclusions

Our study proved that addition of the urban bottom sediment to the soil might result in plant toxicity. However this inhibition in growth is plant species-specific. The higher resistance of *F. arundinacea* confirms that this species could be a useful candidate for remediation of contaminated sites, particularly those contaminated with PAHs and PCDD/PCDFs. The observed phytotoxicity in *Tagetes* was likely due to the cumulative effect of all contaminants: PAHs, PCDD/PCDF and possibly some TE. In contrast to what was observed in plants, the sediment did not inhibit microbial activity, estimated by the soil enzymatic activity.

Potential toxicity of urban bottom sediments to ornamental plants suggests that their utility as soil amendment within green urban areas is limited. However their potential use for reclamation purposes, when the target plants are grasses, should not be discarded and further assessments should be carried out to determine the optimal sediment:soil ratio and plant species. Moreover, this study revealed a high potential for application of selected plant growth-promoting

bacterial strains to alleviate soil toxicity and promote plant growth and performance. It also suggests that these bacterial inoculants could be incorporated into phytoremediation strategies for the treatment of contaminated sediments. It would also be interesting to carry out further studies combining plant growth-promoting bacterial strains, such as those identified here, with organic contaminant-degrading strains.

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