Role of *Chlorella* sp. and rhamnolipid 90 in maintaining homeostasis in soil contaminated with bisphenol A

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Abstract

Purpose The knowledge about the impact of BPA on soil health does not correspond to the great interest in its analogues. Therefore, a study was conducted to determine the potentially negative impact of BPA on the biochemical properties of soil. The study also evaluated the effectiveness of two biostimulants in eliminating potential homeostasis disorders caused by BPA.

Materials and methods A pot experiment was conducted under supervised conditions. BPA at five contamination levels was added to the soil of the granulometric composition of sandy loam at 0, 0.1, 2, 40 and 800 mg BPA kg⁻¹ of a dry matter (DM) of soil. The biochemical activity of the soil was interpreted through the activity of dehydrogenases (Deh), urease (Ure), catalase (Cat), acid phosphatase (Pac), alkaline phosphatase (Pal), arylsulphatase (Aryl) and β -glucosidase (Glu) whose activity was determined on days 5, 15 and 45 of the study. The biostimulative potential of *Chlorella* sp. and rhamnolipid 90 (which eliminates the undesirable effects of BPA on the parameters) was expressed with IF_B—the factor of the impact of increasing of bisphenol (BP) soil contamination levels. The response of spring barley to increasing BPA pressure was analysed with the plant resistance index (RS). The study was made more comprehensive by determination of the macronutrient content in the plants.

Results and discussion The sensitivity of individual enzymes to increasing bisphenol pressure on the 45th day of the experiment can be arranged in the following sequence: Deh > Ure > Glu > Pac > Cat > Aryl > Pal. Biostimulation of soil with *Chlorella* sp. gave better results than with rhamnolipid 90. A compilation of BPA 800 mg BPA kg⁻¹ DM of soil and *Chlorella* sp. brought about an increase in the activity of Glu on the 45th day of the experiment and Pac, Pal and Aryl on the 5th day. Only at this contamination level did BPA stimulate the crop growth in all the parallel plots except in those biostimulated by *Chlorella* sp. Only algae significantly reduced the negative BPA impact on the N, Ca and K content in spring barley.

Conclusions The experiment emphasised the significant inhibitory impact of BPA on the biochemical activity of soil which, in consequence, upset the microbial balance of soil processes. *Chlorella* sp. played a more important role in maintaining the soil homeostasis than rhamnolipid 90, which did not correspond to its negative impact on the yield of spring barley.

Keywords BPA · Soil · Enzymes · *Chlorella* sp. · Rhamnolipid 90

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1 Introduction

Bisphenol A (BPA) is regarded as the most widely produced chemical compound worldwide (Yu et al. 2015). The chemical structure of BPA is based on dihydroxybenzene with two hydroxyl groups in the para positions, which determines its good reactivity. BPA can be transformed into ethers, esters and salts and undergoes electrophilic substitution, such as sulphonation, alkylation and nitration (Flint et al. 2012). Moreover, its water-octanol coefficient in the logarithmic form ($\log P = 3.32$) indicates that it is readily soluble in fats and poorly soluble in water: ca. 200 mg dm⁻³ at 25 °C (Vandenberg et al. 2012).

The global demand for BPA in 2015 amounted to approx. 7.70×10^9 kg. Currently, the annual production output or import of BPA in the European Economic Area amounts to $1-10 \times 10^9$ kg, and it is expected to increase to 10.60×10^9 kg by 2022 (Cyzdik-Kwiatkowska et al. 2017; ECHA 2019). The greatest average increase in the global BPA production output is observed in India. It is associated with a 19% increase in demand for polycarbonates in this country (Bisphenol A 2016).

BPA is used in the production of multiple consumer goods (Guerra et al. 2015), including thermal paper, dental sealers (Pookpoosa et al. 2015), food containers, bottles and toys (Huang et al. 2012; Hu et al. 2019). BPA has also been reported as a semi-finished product in the manufacturing of dyes for fabrics (Xue et al. 2017). It is also widely used in the production of polyacrylate coats and can paints (Vandenberg et al. 2012). BPA analogues are degradation products of the pesticide-methoxychlor, used in some developing countries (Gupta et al. 2006). Bisphenol AF (a fluorinated BPA analogue) is synthesised as a monomer for partially fluorinated polycarbonates (Yang et al. 2014). Tetrabromobisphenol A (TBBPA) is referred to as a flame retardant in manufacturing electronic equipment (Wang et al. 2016). Copolyesters of AxPET/FxPET, with an addition of bisphenol A and bisphenol F, have been found to be more thermostable and a better flame retardant than pure PET (De-Ming et al. 2015).

Increased BPA migration to the environment is caused, among others, by the production and processing of BPA and by polymer hydrolysis, which results in releasing BPA monomers to ecosystems and to food (Mercea 2009). According to Fenichel et al. (2013), BPA disrupts the oestrogen system, but also the function of androgens, prolactin, insulin and thyroid hormones. Through various cellular signalling pathways, BPA induces carcinogenesis and foetal malformations in the brain and the nervous system (Murata and Kang 2018). It is responsible for improper activation and AR-T877A mitogenesis in prostate cancer cells (Lee et al. 2003). In ovarian cells and breast cancer cells, it induces activation of protein kinase regulated by the $\frac{1}{2}$ signal (MARK/ERK1/2) (Song et al. 2015).

Growing concerns about BPA safety have been exacerbated by reports on the compound distribution in the environment. BPA is emitted to the atmosphere mainly as a result of industrial activities. These parameters are estimated at 100×10^9 kg annually. The highest average global BPA concentrations (4.55 ng m^{-3} of air) were recorded in the urban regions of India, which corresponded to intensive burning of plastic products for household purposes (Fu and Kawamura 2010). Common use of BPA also contributed to the distribution of the compound in the aquatic environment, mainly by effluents from landfills and wastewater treatment plant discharges (Morin et al. 2015). Ultimately, it resulted in the contamination of freshwater and marine waters with this bisphenol

(Couderc et al. 2015). Rocha et al. (2013) found BPA in half of Portuguese rivers in concentrations ranging from 28.7 to 98.4 ng BPA dm^{-3} of water. Sewage sludge is also an important source of BPA (Flint et al. 2012). According to Song et al. (2014), the BPA detection rate for sludge in China was 76.9% and 97.5% in sludge in Korea, and it reached 100% in sludge in America (Lee et al. 2015; Yu et al. 2015). It must be stressed that bisphenols are more durable in sludge ($t_{1/2} = 135-1621$ days) than in soil ($t_{1/2} = 30-360$) (Chen et al. 2016). Their degradation is correlated positively with the soil pH and TOC (Kalmykova et al. 2013). Bisphenol transformations in soil between the dissolved phase in soil and the colloidal phase are also affected by iron oxides (Urase and Miyashita 2003). Bisphenol interactions with elements of the soil environment are complex. Kwak et al. (2018) proposed critical value of BPA for protection of the soil ecosystem of 74.70 mg kg^{-1} DM of soil using the species sensitivity approach. However, research in which the BPA toxicity for plants was determined even at the level of $1000 \text{ mg BPA kg}^{-1}$ DM of soil contamination (Kim et al. 2018) was conducted. One of the manifestations of its complex nature is the process of BPA glycosylation induced in plants, which produces secondary metabolites of mono- and di-O- β -d-glucopyranosides without the oestrogen activity (Zaborowska et al. 2017; Nakajima et al. 2002).

Obviously, BPA biodegradation is also a consequence of bisphenol use by some bacteria as the only energy source, although they inhibit the growth of many strains (Kolvenbach et al. 2014). Two main mechanisms of BPA biodegradation occurring under aerobic conditions have been identified. The first is based on the oxidative rearrangement of the skeleton of an aliphatic methyl group in a bisphenol molecule. It has been shown for *Cupravidus basilensis* JF1 (Fischer et al. 2010). *Sphingobium xenophagum* is capable of metabolizing BPA by an ipso-substitution mechanism that involves ring hydroxylation at the site of the substituent (Gabriel et al. 2007). This is the second major BPA biodegradation pathway that involves the hydroxylation of one or two phenol rings followed by cleavage of the aromatic ring. It was demonstrated also for *Sphingobium fuliginis* OMI (Ogata et al. 2013). The diverse responses of microorganisms to BPA are caused by the expression of various genes: H6PD inducing the pentose phosphate pathway, CTH participating in the cysteine metabolic process, TRIM66 responsible for regulating transcription and PPP1R3E inducing the glycogen metabolism process (Yin et al. 2014). *Pseudomonas aeruginosa* has rhlC, rhlAB and PA1131 genes required for rhamnolipid synthesis (Bazire and Dufour 2014; Wittgens et al. 2017). According to Furmańczyk et al. (2017), *Pseudomonas umsongensis* has genes rfbADB and rfbC which encode proteins responsible for the synthesis of rhamnolipid, which is a biosurfactant effective in hydrocarbon degradation and which facilitates their emulsification. It also produces a gene HHDH-

Pu which encodes halohydrin dehalogenase, which is responsible for enzymatic opening of the epoxide ring (Xue et al. 2018). The aerobic catabolism of bisphenols is catalysed by monooxygenases, phenol hydroxylase, which adds one atom from an oxygen molecule to an aromatic ring, and toluene/o-xylene monooxygenase (TOMO), capable of hydroxylation of more than one position of the aromatic ring (Cafaro et al. 2004). Furthermore, algae consume carbon dioxide for their growth, thereby inducing BPA transformation to derivatives of non-oestrogenic potential (Hirooka et al. 2005; Zaborowska et al. 2017).

To date, in-depth analyses have covered the microorganisms' BPA degradation potential rather than soil enzyme response to contamination of the soil environment with this compound, although soil enzymes are regarded as reliable indicators of soil biological quality representing their metabolic potential. However, the first reports have appeared on the impact of BPA, bisphenol S (BPS) and bisphenol F (BPF) analogues on soil biochemical and microbiological activity (Zaborowska et al. 2019, 2020a, b). BPS and BPF are considered less controversial substitutes for BPA harmful to humans. The premise for choosing BPS and BPF for research was the growing interest in these phenolic compounds. However, it does not correspond with the amount of research carried out on the impact of these bisphenols on the biochemical activity and response of soil microorganisms. It turned out that both BPS and BPF are potent inhibitors of both soil enzyme activity and the number and diversity of microorganisms. Therefore, the conducted experiment is an important complement to existing research, because there should be awareness of the differences in the BPA, BPS and BPF toxicity for both microorganisms and soil enzymes.

It was therefore regarded as reasonable to analyse the soil enzyme response, referred to as reliable soil quality indicators, to the pressure generated by increasing BPA levels. Preventive actions were also taken in order to offset the potential inhibitory action of bisphenol by soil biostimulation with *Chlorella* sp. and rhamnolipid 90. According to Ji et al. (2014), both *Chlorella mexicana* and *Chlorella vulgaris* can be used to treat the BPA-contaminated aquatic ecosystems. The high bioaccumulation and then the biodegradation rates of 24% are correlated with the content of fatty acids and carbohydrates. The potential of *Chlorella* sp. was determined mainly in soils contaminated with heavy metals (Nam et al. 2019). The impact of an algae in soils contaminated with bisphenols has not been studied so far. The choice of rhamnolipid 90 was dictated by the fact that, on the one hand, *Pseudomonas aeruginosa* produces rhamnolipid biosurfactants involved in numerous processes including removal of phenols and heavy metals from wastewater (Verma and Sarkar 2017), and on the other hand, metabolic pathways were identified, which are induced by *Pseudomonas* sp. and which effectively degrade bisphenols (Singh et al. 2018). The

holistic nature of the study was achieved by determination of the impact of BPA on the growth and development of spring barley. Spring barley is the cereal with the fourth largest global production output, after maize, wheat and rice (Shen et al. 2016; FAOSTAT 2017), and it satisfies approx. 50% of the demand for calories globally (OECD 2017). It also has relatively simple diploid genetics (Sreenivasulu et al. 2008), which increases its importance to science.

2 Material and methods

2.1 Characteristics of BPA

Bisphenol A (BPA) (synonyms: 4,4'-isopropylidenediphenol; 2,2-bis(4-hydroxyphenyl)propane), a white, crystalline substance with a purity of $\geq 99.0\%$ (HPLC) (Sigma Aldrich) was used in the experiment. Selected physical and chemical properties of BPA are presented in Table 1 (Hu et al. 2019; Michałowicz 2014).

2.2 Soil material

The experiment was conducted on soil collected from the Olsztyn Lake District situated in the north-east of Poland, within the province of the Eastern Baltic-Belarusian Lowland, which is part of the Eastern European Plain (NE Poland, 53.72 N, 20.42 E). Since it was the area of the Teaching and Experimental Centre in Tomaszkowo, the soil material before being collected was used as agricultural soil for cereal cultivation in accordance with the practices corresponding to the temperate warm transitional climatic zone in which the area is situated. Proper brown soil with the granulometric composition of sandy loam, determined in accordance with the IUSS Working Group WRB (2014), was collected from the genetic level Ap. Its physicochemical and biochemical parameters were determined by the methodology described by Borowik et al. (2017). Since pH in 1 Mol dm⁻³ KCl was 5.60, CaCO₃ in the amount sufficient to neutralise the hydrolytic acidity of 1.5 (HAC) was added to the soil on day 1 of the experiment. The other soil parameters were the following: hydrolytic acidity (HAC) 6.40 mM(+) kg⁻¹ DM of soil, base saturation (BS) 96.29%, total base exchangeable cations (EBC) 165.90 mM(+) kg⁻¹ DM of soil, total organic carbon (C_{org}) 6.40 g kg⁻¹ DM of soil and total exchangeable cations: K⁺ 180, Na⁺ 20, Ca²⁺ 2571 and Mg²⁺ 59.50 mg kg⁻¹ DM of soil. Since the enzymatic activity and the granulometric composition are components of soil process simulation models, Table 2 presents the selected biochemical properties of the soil.

Table 1 Determination of soil enzyme activity

No.	Enzyme	Substrate	Unit/product	Wavelength (λ) nm	References
1	Dehydrogenases (EC 1.1)	2,3,5-triphenyl tetrazolium chloride (TTC)	μMol triphenyl formazan (TFF) kg^{-1} of soil h^{-1}	485	Öhlinger (1996)
2	Catalase (EC 1.11.1.6)	H_2O_2 -aqueous solution	$\text{Mol O}_2 \text{ kg}^{-1}$ DM of soil h^{-1}	–	Alef and Nannipieri (1998)
3	Urease (EC 3.5.1.5)	Urea -aqueous solution	$\text{mMol N-NH}_4 \text{ kg}^{-1}$ DM of soil h^{-1}	400	
4	β -Glucosidase (EC 3.2.1.21)	4-Nitrophenyl- β -D-glucopyranoside (PNG)	$\text{mMol 4-nitrophenol (PN) kg}^{-1}$ DM of soil h^{-1}	410	
5	Acid phosphatase (EC 3.1.3.2)	disodium-4-nitrophenylphosphate hexahydrate (PNP)		400	
6	Alkaline phosphatase (EC 3.1.3.1)			400	
7	Arylsulphatase (EC 3.1.6.1)	Potassium-4-nitrophenyl -sulfate (PNS)		420	

2.3 Characteristics of biostimulants

Chlorella sp. is unicellular green algae of class *Trebouxiophyceae* (Nam et al. 2019). The content of nitrate

nitrogen ($\text{NO}_3\text{-N}$) and ammonium nitrogen ($\text{NH}_4^+\text{-N}$) was 0.03 g kg^{-1} DM and 9.81 g kg^{-1} DM, respectively. The macronutrients content expressed in mg kg^{-1} DM of *Chlorella* sp. was N 95.3, P 9.9, K 9.0, Mg 4.5 and Ca 4.7. Rhamnolipid 90

Table 2 Enzymatic activity in soil contaminated with BPA in the 5th, 15th and 45th days of research, kg^{-1} DM of soil h^{-1}

Dose of BPA (mg kg^{-1})	Deh ($\mu\text{Mol TFF}$)	Cat (Mol O_2)	Pac ($\text{mMol 4-nitrofenol PN}$)	Pal	Aryl	Glu	Ure (mMol N-NH_4)
5th day							
0	1.39 ^c	0.13 ^c	4.75 ^c	0.79 ^a	0.01 ^b	0.25 ^a	0.78 ^a
0.1	1.46 ^{bc}	0.18 ^{ab}	4.84 ^b	0.68 ^b	0.02 ^a	0.29 ^a	0.45 ^b
2	1.69 ^b	0.18 ^a	4.96 ^a	0.54 ^c	0.02 ^a	0.29 ^a	0.42 ^b
40	1.68 ^b	0.16 ^{bc}	4.65 ^d	0.56 ^c	0.01 ^b	0.29 ^a	0.25 ^c
800	2.31 ^a	0.15 ^c	4.68 ^{cd}	0.47 ^d	0.01 ^c	0.27 ^a	0.24 ^c
<i>r</i>	0.82*	−0.14	−0.40	−0.95*	−0.61*	−0.41	−0.92*
15th day							
0	1.17 ^d	0.17 ^{bc}	4.57 ^{ab}	0.55 ^c	0.01 ^b	0.43 ^a	0.32 ^{ab}
0.1	2.71 ^b	0.18 ^b	4.67 ^a	0.59 ^c	0.02 ^b	0.32 ^b	0.27 ^{ab}
2	2.89 ^b	0.15 ^c	4.65 ^a	0.77 ^a	0.01 ^b	0.37 ^{ab}	0.24 ^b
40	3.30 ^a	0.17 ^{bc}	4.56 ^{ab}	0.71 ^b	0.01 ^b	0.37 ^{ab}	0.31 ^{ab}
800	1.77 ^c	0.29 ^a	4.46 ^b	0.78 ^a	0.02 ^a	0.36 ^b	0.35 ^a
<i>r</i>	0.32	0.64*	−0.64*	0.87*	0.57*	−0.33	0.35
45th day							
0	5.18 ^a	0.21 ^b	4.93 ^{bc}	0.69 ^d	0.01 ^b	0.37 ^{bc}	0.99 ^b
0.1	4.62 ^b	0.15 ^c	5.07 ^{ab}	0.67 ^d	0.01 ^b	0.39 ^b	1.15 ^a
2	4.61 ^b	0.16 ^c	5.15 ^a	0.72 ^c	0.01 ^b	0.45 ^a	0.82 ^c
40	3.65 ^d	0.17 ^c	5.08 ^{ab}	0.7 ^{a9}	0.01 ^b	0.34 ^c	0.79 ^c
800	4.11 ^c	0.31 ^a	4.75 ^c	0.75 ^b	0.04 ^a	0.33 ^c	0.77 ^c
<i>r</i>	−0.85*	0.54*	−0.34	0.81*	0.65*	−0.40	−0.77*

Homogeneous groups specified in columns, for each enzyme, depending on the increasing doses of BPA during the 5, 15 and 45 days of experiment

Deh dehydrogenases, *Cat* catalase, *Ure* urease, *Pal* alkaline phosphatase, *Pac* acid phosphatase, *Glu* β -glucosidase, *Aryl* arylsulfatase, *r* Pearson's linear correlation coefficient

*Significant for $P = 0.05$, $n = 14$

(No. CAS 869062-42-0) is a glycolipid synthesised by *Pseudomonas aeruginosa*, containing L-ramnose and β -hydroxy amphiphilic fatty acids (*Sigma Aldrich*). Its application rate is based on the critical concentration of rhamnolipid micelles (CMC) (Renfro et al. 2014).

2.4 Design of pot experiment

Analyses of soil enzyme responses to the pressure of increasing soil contamination with BPA have been scarce. Therefore, the choice of the method of conducting the experiment played a key role in obtaining reliable study results, unaffected by variables that could potentially modify the existing correlations. Therefore, the experiment was conducted under monitored conditions, in five replicates, on properly prepared soil material. Application to the soil of a sequence of experimental variables was preceded with soil fertilisation suitable for the specific crop. The form of fertilisation is described by Zaborowska et al. (2017) and the fertilisation rate, expressed as a pure nutrient, was N 250, P 50, K 90, Mg 20, Cu 5, Zn 5, Mo 5, Mn 5 and B 0.33 (mg kg⁻¹ of soil). The potential inhibition was analysed based on four major factors: (1) the degree of soil contamination with BPA: 0, 0.1, 2, 40, 800 mg BPA kg⁻¹ DM of soil; (2) experiment duration depending on the crop growth phase: 5 days, 15 days—tillering phase (BBCH 21—beginning of the tillering phase: first tiller detectable), 45 days—heading phase (BBCH 52–20% of inflorescence emerged); (3) addition of potential biostimulants: rhamnolipid 90 (0 and 150 mg kg⁻¹ DM of soil) and *Chlorella* sp. (0 and 5 g kg⁻¹ DM of soil) and (4) method of soil use: unsown soil and soil sown with spring barley (*Hordeum vulgare* L.) var. *Orphelia*. A total of 45 combinations were analysed. The activity of soil enzymes was determined in soil samples taken from 15 research objects on days 5, 15 and 45 during the spring barley growth. The biostimulation scale of *Chlorella* sp. and rhamnolipid 90 was also determined in each of the three test dates. The tests were carried out in four replications. They also included uncontaminated BPA control objects. One kilogram of soil was packed into each polyethylene pot. Assumptions of the experiment performed under controlled conditions allowed monitoring of soil humidity that was kept at the level of 60% using distilled water. Day time ranged from 13 h 3 min to 16 h 31 min. The average air temperature was 15.6 °C, and air humidity was 76.5%. In the next step, 15 seeds of spring barley were sown in selected pots, and after 9 days (BBCH 9—coleoptile penetrates soil surface), five plants were left. Spring barley was harvested on day 45, and its fresh and dry weights were determined as well as BPA content in the above-ground parts of the plants.

2.5 Determination of soil enzyme activity

The activity of seven enzymes: dehydrogenases, urease, catalase, acid phosphatase, alkaline phosphatase, arylsulphatase and β -glucosidase was determined in each soil sample in three

replicates, by methods specified in Table 1. The biochemical activity of the soil was determined with a Perkin-Elmer Lambda 25 spectrophotometer (Massachusetts, USA). Catalase was an exception; its main function at a high concentration of hydrogen peroxide is to participate in its decomposition to water and oxygen (Tehrani and Moosavi-Movahedi 2018). Therefore, its activity was determined by titration of hydrogen peroxide residue. The determinations were performed on days 5, 15 and 45 of the experiment.

2.6 Determination of the chemical composition and BPA residue in spring barley

In order to analyse the crop response to the BPA pressure, the study involved determination of the content of macronutrients: N, P, K, Mg and Ca in the dry weight of the spring barley above-ground parts and the BPA residue in pots contaminated with 800 mg BPA kg⁻¹ DM of soil. The macronutrients were determined after oxidising wet pre-mineralisation with sulphuric acid (98%). The Kjeldahl method (N_{total}) and the UV-VIS spectrophotometric method (P) with flame photometry (K) and atomic absorption spectrometry (AAS) (Mg and Ca) were applied (Sivitskaya and Wyszowski 2013). The BPA residue in barley was determined by gas chromatography coupled with tandem mass spectrometry on a Bruker Scion TQ “triple quadrupole” gas chromatograph and the monitoring of selected reactions (GC-MS/MS) following the sample ultrasound-aided extraction with acetonitrile and derivation with a mixture of N, O-bis(trimethylsilyl)trifluoroacetamide and 5% trimethylchlorosilane. The determinations were performed at the measurement sensitivity of 0.1 mg BPA kg⁻¹ DM of soil, ion source temperature 200 °C, dosing temperature 280 °C, ionization type EI 70 eV and expansion factor $k = 2$, with a 95% confidence level. The determinations were made at the PORT Institute Polish Center for Technology Development in Wrocław.

2.7 Computations and statistical analysis

All of the statistical analyses were conducted with Statistica 10.0 software (StatSoft Inc. 2018). This experiment involved determination of the variance percentage of the variable under analysis (η^2) defined by the analysis of variance—ANOVA—and changes in the macronutrient N, P, K, Mg and Ca content in the crop and the response of each enzyme to the soil contamination with BPA and biostimulation with the principal component analysis—PCA. Homogeneous variances between the enzymes were illustrated by Tukey’s test (HSD) at $P = 0.01$, and the cluster method—a dendrogram by the Ward method—which grouped the enzymes with respect to their sensitivity to BPA. It was configured based on the BPA impact factor (I_{BP}) calculated from the formula:

$$IF_{BP/b(bC/bR)} = \frac{A_{BP/B}}{A_C}$$

where

IF_{BP} —the factor of the impact of increasing bisphenol (BP) soil contamination levels, ($IF_{BP} < 1$ —inhibition of the enzyme activity by BPA, $IF_{BP} > 1$ —stimulation of the soil enzyme activity by BPA; A_{BP} —enzyme activity in the soil subjected to the increasing BPA contamination pressure; A_C —activity of the enzyme in the control soil non-contaminated with BPA. The biostimulation potential of the *Chlorella* sp. and rhamnolipid 90 was determined using the ($IF_{bC(bR)}$) index where $IF_{bC(bR)}$ —factor of the *Chlorella* sp. (IF_{bC}) and rhamnolipid's 90 (IF_{bR}) impact, $IF_{bC(bR)} < 1$ —*Chlorella* sp. and rhamnolipid 90 inhibit the enzyme activity, $IF_{bC(bR)} > 1$ —*Chlorella* sp. and rhamnolipid 90 stimulate the enzyme activity; A_B —enzyme activity in the soil biostimulated with *Chlorella* sp. and rhamnolipid 90 and subjected to the increasing BPA contamination pressure; A_C —activity of the enzyme in the control soil and biostimulated with the *Chlorella* sp. and rhamnolipid 90.

Spring rape resistance (RS) to increasing BPA soil contamination was determined using the Orwin and Wardle's formula (2004) described by Zaborowska et al. (2019).

3 Results

3.1 Activity of soil enzymes

These analyses revealed a varied response of soil enzymes to increasing BPA pressure. The response of individual soil enzymes to bisphenol was reinforced by the analysis of variance (Fig. 1). The dose proved to be the test factor that had the greatest impact on the changes in Pac, Ure and Aryl activity, respectively: 15.28%, 8.16% and 7.30%, as opposed to Deh (0.05%). Furthermore, time was a significant moderator in transformations of Glu, Pac and Aryl, and η^2 above 20% were 46.84%, 32.31% and 23.05%, respectively. Upsetting the biochemical balance on day 5 of the study resulted mainly from BPA inhibition with respect to Pal ($r = -0.949$) and Ure ($r = -0.917$), which is indicated by the homogeneous groups corresponding to the correlation coefficients (Table 2). Fifteen-day exposure to BPA contributed to stimulation of the Cat, Aryl and Deh activity. It must be emphasised that BPA at the lowest contamination level (0.1 mg kg⁻¹ DM) induced two-fold higher activity of dehydrogenases. An assessment of the soil homeostasis through Deh and Ure showed its considerable disruption on day 45 of the experiment, following the application of 40 mg BPA kg⁻¹ DM of soil. The enzyme activity decreased under their pressure by 30% and 20%, respectively. The cluster analysis diagrams plotted by the Ward's method concerning the I_{BP} index show the revealed

relationships (Fig. 2a, b, c). The enzyme response to the highest contamination level of 800 mg BPA kg⁻¹ DM of soil following 5 days of exposure to the bisphenol generated two clusters of homogeneous variances (Fig. 2a). Ure, Aryl and Pal (cluster I) and Deh, Pac, Cat and Glu (cluster II) were assigned to them. Lower values of I_{BP} were calculated for the enzymes in cluster I. The diagram of biochemical transformations in the soil exposed to BPA for 15 days showed the different response of Deh to increasing soil contamination with BPA than the other enzymes (cluster III), although it emphasised the significant stimulation of Deh, Cat and Aryl in the soil exposed to a pressure of 800 mg BPA kg⁻¹ DM (cluster II) (Fig. 2b). The BPA impact index I_{BP} values were high in these pots: I_{BP} Deh = 1.582, I_{BP} Cat = 1.707, I_{BP} Aryl = 1.496. The biotic stress caused by soil contamination with BPA for 45 days placed Ure, Deh and Glu in a separate cluster (II) of homogeneous variances, which is justified by the sensitivity of the enzyme to soil contamination levels exceeding 40 mg BPA kg⁻¹ DM (Fig. 2c). It is noteworthy that there is a separate subcluster (I) which shows the arylsulphatase response to the application of 800 mg BPA kg⁻¹ DM of soil. The compilation of the time and bisphenol dose contributed to the Aryl $I_{BP} = 2.646$.

3.2 Biostimulation with *Chlorella* sp. and rhamnolipid 90

High values (η^2) for the extent of biostimulation with *Chlorella* sp. and rhamnolipid 90 of soil contaminated with BPA confirmed that this experimental factor is highly significant in biochemical transformations of the soil environment under study (Fig. 1). Biostimulation moderated the activity of all enzymes in the following sequence: Cat (81.28%) > Pal (54.91%) > Deh (47.03%) > Ure (27.08%) > Aryl (16.85%) > Pac (10.23%) > Glu (5.43%). The effectiveness of the substances used was determined with multidimensional PCA analysis (Figs. 3 and 4). The existing tendencies were determined with the biostimulation impact factor IF_B . The first variable explaining 44.91% of the total data variance clustered the ends of vectors corresponding to IF_{BCh} for Deh, Ure, Pac, Pal and Cat. Furthermore, the variable explaining 21.18% of the variable variance induced the distribution of the ends of vectors for IF_{BCh} for Glu and Aryl (Fig. 3). The case dislocation proved that *Chlorella* sp. effectively increased Glu activity in pots contaminated with 40 and 800 mg BPA kg⁻¹ DM of soil on day 45 of the experiment. A compilation of the largest dose of BPA (800 mg kg⁻¹ DM of soil) and *Chlorella* sp. on day 5 of the experiment stimulated the activity of Pac, Pal and Aryl and, likewise, the algae played their role in the soil under the pressure of the 20-times lower contamination with BPA, increasing the activity of Ure, Deh and Cat. The PCA also highlighted the range of BPA impact and biostimulation with rhamnolipid 90, which was effective for only 15 days of the

Fig. 1 The share of independent variables in the evolution of the soil enzyme activity (η^2): d dose of BPA, b biostimulation, t date of analysis, Deh dehydrogenases, Ure urease, Pac acid phosphatase, Pal alkaline phosphatase, Cat catalase, Aryl arylsulphatase, Glu β -glucosidase (three-way analysis of variance, ANOVA, at $P < 0.05$)

experiment (Fig. 4). This is demonstrated by the coordinates of cases and standardised vectors representing the IF_{BR} and affected by the principal components PCA1 and PCA2. The first principal component generated negative values of vector ends for Aryl (-0.518) and Glu (-0.304) and the second principal component for Deh (-0.459) and Pal (-0.235). The biosurfactant contributed to a positive response of Deh to the highest level of contamination ($800 \text{ mg BPA kg}^{-1} \text{ DM}$ of soil) after 15 days of exposure to bisphenol applied to the soil. Biostimulation of soil contaminated with $40 \text{ mg BPA kg}^{-1} \text{ DM}$ of soil also induced an increase in the activity of Cat, Ure and Pac (day 5 and 15) and Aryl and Glu (day 45).

3.3 The effect of BPA on spring barley

Since bisphenols are referred to as “priority substances” placed on ATSDR’s Substances Priority List (ATSDR’S 2017) based on their toxicity and occurrence, it was important to verify as many factors as possible whose stable functions were disrupted by BPA. Therefore, the study characterised the spring barley resistance index to BPA in soil (Fig. 5). A negative correlation was found between the soil contamination with the bisphenol increasing to $40 \text{ mg BPA kg}^{-1} \text{ DM}$ of soil and the crop resistance. Biostimulation with *Chlorella* sp. and rhamnolipid 90 did not bring any desired effects and even exacerbated the growth and development inhibition of spring barley in the case of algae. It is intriguing that the crop yield was significantly stimulated both in control pots with the highest level of contamination with BPA ($800 \text{ mg kg}^{-1} \text{ DM}$ of soil) and in parallel pots with rhamnolipid 90. The study

scope was expanded to include an assessment of the BPA impact on the macronutrient content in spring barley. It was performed by means of a principal component analysis (Fig. 6). The distribution of all the cases on the PCA map illustrated the beneficial impact of *Chlorella* sp. on the content of N, K and Ca in a plant, which was not observed in the control pots or in those biostimulated with rhamnolipid 90. This benefit is also demonstrated by positive values of the variable ends representing N, K and Ca with respect to the second principal component (PCA2) describing 16.20% of variable variance.

4 Discussion

4.1 Soil enzymes

The sensitivity of the soil enzymes to increasing BPA pressure changed over the course of the experiment. On the 5th day, the phenolic compound applied to the soil proved to be the most potent inhibitor of Pac, Ure and Aryl. On the 15th day, the inhibitory effects of BPA on Pac, Glu and, to a lesser extent, Deh were found. In turn, on the 45th day of the experiment, Deh, Ure and Glu proved to be the most sensitive to BPA. Biochemical activity is included in simulation models for soil processes, correlated with the organic matter decomposition rate (Wyszkowska et al. 2017; Kucharski et al. 2016). Dehydrogenases are regarded as a reliable bioindicator of xenobiotic toxicity (Campos et al. 2019). Soil dehydrogenases are reliable to indicate xenobiotic toxicity because they occur

Fig. 2 Similarity of soil enzymes reaction to contamination of soil with BPA based on the coefficient of impact BPA (I_{BP}) in 5th (a), 15th (b) and 45th (c) day of research; Deh dehydrogenases, Cat catalase, Ure urease, Pal alkaline phosphatase, Pac acid phosphatase, Glu β -glucosidase, Aryl arylsulfatase; doses of BPA mg kg^{-1} DM of soil: 0.1, 2, 40, 800

in all living cells of microorganisms. They are also characterized by a lack of ability to accumulate in the extracellular environment (Zhan et al. 2010). The fact that dehydrogenases carry out dehydrogenation or hydrogenation processes makes them relevant in the enzyme systems of all living microorganisms. In addition, soil dehydrogenases can use not only oxygen molecules as an electron acceptor but also other compounds that occur in the cells of anaerobic microorganisms

(Brzezińska et al. 2001). Since the study conducted by Zaborowska et al. (2019) found them to be the most sensitive to the pressure of bisphenol S, which is a BPA analogue, a similar response was expected. On the other hand, dehydrogenases participate in the dehydrogenation of phenolic compounds and ethylbenzene conversion to 1-phenylethanol, and the ultimate acetophenone degradation to benzaldehyde and benzoic acid (Daudzai et al. 2018) which,

Fig. 3 Coefficients of impact (IF_{bC}) of *Chlorella* sp. for enzymes activity in soil contamination with BPA-PCA method; black square—the end of the vector of the primary variable: Deh dehydrogenases, Cat catalase, Ure urease, Pac alkaline phosphatase, Aryl arylsulfatase; black circle cases: doses of BPA mg kg^{-1} DM of soil: 0, 0.1, 2, 40, 800; time: *I*—5th day, *II*—15th day, *III*—45th day of research

in turn, makes one put forward different hypotheses correlated to this study. Moreover, according to Herter et al. (2011), the intensity of phenolic compound inhibition is significantly affected by the presence and position of selected substituents. Researchers have put phenolic compound derivatives in the following sequence based on the extent of phenol oxidase activity stimulation: 2,6-dimethoxyphenol > ABTS > ortho-dihydroxylated compounds > monoethoxylated monophenols > dimethoxylated monophenols > para-dihydroxylated compounds. A similar relationship was observed for urease. N^1, N^2 -diaryl derivatives containing nitro groups in phenyl rings exhibited low-protein urease inhibition (Perveen et al.

2008). Furthermore, organic compounds containing the methoxy group or hydroxy groups in the phenyl ring were found to be more toxic to urease (Mustafa et al. 2014; Rajic et al. 2009), which corresponds to the results of the current study. Khadem and Raiesi (2019) observed an opposite relationship. The presence of carboxyl and hydroxyl groups contributes to adsorption of alkaline phosphatase on soil colloids by interacting with amino groups in the enzyme molecules, thereby inducing its activation. Furthermore, the list of catalase inhibitors contains flavonoids (Krych et al. 2014). However, the peroxidatic pathway is induced at low concentrations of H_2O_2 in the soil environment, in which phenol

Fig. 4 Coefficients of impact (IF_{bR}) of rhamnolipid 90 for enzymes activity in soil contaminated with BPA-PCA method (for abbreviations, see Fig. 5)

Fig. 5 The content of macronutrients N, P, K, Ca, Mg by spring barley (*Hordeum vulgare* L.)—PCA method; black square—the end of the vector of the primary variable; black circle cases: 0, 800—doses of BPA kg^{-1} DM of soil, C control, CH *Chlorella* sp., R rhamnolipid 90

assumes the role of hydrogen peroxide and becomes the hydrogen donor (Hong et al. 2012).

It should also be taken into account that the functional viability of enzymes in mineral soils, as a result of reaction with minerals, lasts much longer in them than in organic soils (Schimel et al. 2017). In the soil environment, enzymes can also be stabilized by interactions with organic matter or metabolised by microorganisms. Repeatedly, soil enzymes undergo thermal denaturation (Burns et al. 2013). Organic compounds serving as food resource for microbes can co-precipitate with Fe or Al cations to form secondary mineral

phases (Fe and Al oxyhydroxides) (Tamrat et al. 2019). In some cases, some microbes can access the organic compounds in organo-mineral associations, but it requires the secretion of specific enzymes whose affinity to the organic compounds is higher than its adsorption affinity (Basile-Doelsch et al. 2020). It should be emphasized that organic matter, pH and Fe oxides have the greatest impact on the exchange of organic chemical compounds between the soil solid phase and the solution phase. At the low pH value, the BPA removal rate decreases. In contrast, an increased BPA degradation efficiency was observed at high pH (Yu et al. 2019). Therefore, an example of

Fig. 6 Index of spring barley resistance (RS) depending on BPA contamination and biostimulation with *Chlorella* sp. and Rhamnolipid 90, 0.1, 2, 40, 800—doses of BPA (mg kg^{-1} DM of soil) and content of BPA in spring barley (mg kg^{-1} DM of barley); C control, CH *Chlorella* sp., R rhamnolipid 90

soils distinguished by a high content of bisphenols is peat soils, which are characterized by low pH value and the litter from peatland vegetation (Wiedermann et al. 2017).

4.2 Biostimulation with *Chlorella* sp. and rhamnolipid 90

The use of *Chlorella* sp. and rhamnolipid 90 brought the intended effect. Both rhamnolipid 90 and *Chlorella* sp. proved to be effective biostimulants of soil biochemical activity. However, *Chlorella* sp. enhanced the activity of individual enzymes more intensively. It contributed most to the increase of Glu, Ure, Deh and Cat activity in soil under pressure of 40 and 800 mg BPA kg⁻¹ DM whereas rhamnolipid 90 had a positive effect mainly on the activity of Deh, Aryl and Glu in soil contaminated with these doses of phenolic compound. According to the recent research (Nam et al. 2019), soil algae (including *Chlorella* sp.) are good bioindicators of soil quality. This can be regarded as an adverse process from the point of view of the accumulation of organic contamination in algae, which is the first step in biomagnification. However, when their potential for biostimulation of the soil biochemical activity is exploited, this property can be seen as an asset. Guo et al. (2017) demonstrated the presence of bound BPA residues defined as non-extractable. After only 10 days of exposure to bisphenol A, algae cells accumulated 15% of it, and the bioconcentration index for total radioactivity in algae was 106 cm³ g⁻¹ DM of algae, which indicates that the biostimulation was effective. On the other hand, algae themselves are an important source of bioactive compounds, including phenols, which have protective properties against abiotic and biotic stress (Onofrejšová et al. 2010). A group of natural BPA metabolites in algae includes halogen derivatives of p-hydroxybenzoic acid, cinnamic acid ester (isopropyl *n*-butyl-3,5-dimethoxy-4-hydroxycinnamate) and florotannins (Hayat et al. 2002). The effectiveness of rhamnolipid 90 can be seen in that it replaces lipopolysaccharides in *Pseudomonas aeruginosa*, which protect them against organic contamination (Zorádová et al. 2011). Li et al. (2018) also demonstrated the adsorptive capability of this group of biosurfactants with respect to phenolic ions because of the benzene ring and polar hydroxyl groups in phenol molecules. According to Liu et al. (2012), the adsorptive capability increased with the soil environment pH between 2 and 7 and decreased in the pH range between 7 and 10. Rhamnolipid induced by *Pseudomonas* Ph6 changes the surface potential of the cell and its hydrophobic properties which, in turn, induces diffusion of hydrophobic organic compounds to cytochylems and their ultimate degradation (Zhong et al. 2008). As reported by

Ma et al. (2018), biodegradation was fostered by rhamnolipid doses of ≥ 100 mg dm⁻³.

4.3 The effect of BPA on spring barley

The reaction of spring barley to the increasing soil contamination by BPA was quite surprising. The resistance of a crop plant decreased with the increase of soil contamination by phenolic compound at the level of 40 mg BPA kg⁻¹ DM of soil. However, stimulation of spring barley growth was observed under pressure from the highest dose of 800 mg BPA kg⁻¹ DM of soil. Although neither *Chlorella* sp. nor rhamnolipid 90 stimulated the growth of spring barley, *Chlorella* sp. exerted a beneficial effect on the content of N, K and Ca in the plant. Based on reports (Shah et al. 2017) on physiological and biochemical response of spring barley to elevated levels of N, one could suppose that the response of Ca and K to a compilation of soil contamination with BPA and biostimulation with *Chlorella* sp. would be similar. Moreover, according to And and Wayne (2003), an elevated Ca²⁺ concentration in cytosol and Ca²⁺ binding with calmodulin (CaM) is a result of barley response to biotic stress, which explains the relationship between the crop low resistance and an elevated concentration of Ca in pots with *Chlorella* sp. It is also noteworthy that Ca takes part in signal transduction for GABA, which initiates phenol accumulation in barley which, in turn, justifies the BPA content elevated in the above-ground parts of barley, as found in this study (Ma et al. 2019). It was found in a study conducted by Alobwede et al. (2019) that *Chlorella* sp. also contributed to an increase in the N content in the soil, which was not correlated with the crop yield. Therefore, the only controversy concerns the interaction between the highest applied dose of BPA (800 mg kg⁻¹ DM of soil), the Mg content and the spring barley yield. Chen et al. (2018) reported that a low Mg content leads to a decrease in chlorophyll biosynthesis by inhibition of expression of genes encoding the Mg-chelatin (ChII) subunit and PPMT encoding Mg-protoporphyrin methyltransferase. Meanwhile, no disturbance in the barley yield was observed following the application of 800 mg BPA kg⁻¹ DM of soil. However, one should not dismiss the fact that plants contain monooxygenases of cytochrome P450 (CYP8D1), which increase their resistance to organic contamination (Daudzai et al. 2018). Furthermore, the activity of biosurfactants can be moderated by phenolic compounds used preferentially as a source of carbon (Pérez-Armendáriz et al. 2013). This was probably the reason why the interaction of BPA at the highest dose and rhamnolipid 90 brought the desired result. At lower levels of contamination with bisphenol A, it may have been disrupted by rhamnolipid activity inhibitors, which include selected soil nitrogen sources, such as glutamine, asparagine or arginine (Venkata-Ramana and Karanth 1989).

5 Conclusions

The experiment highlighted the negative impact of BPA on soil biochemical activity and, in consequence, disturbing the microbial balance of soil processes. The response of individual soil enzymes was varied. The duration of the experiment was an important moderator of soil enzymatic activity. On the 5th day, Pal turned out to be the most sensitive to BPA pressure. On the 15th day, scale of BPA inhibition was the largest for Pac. However, on the 45th day, BPA was proved to be the strongest Deh and Ure inhibitor. *Chlorella* sp. proved to be a better biostimulant of soil enzymatic activity than rhamnolipid 90. The use of *Chlorella* sp. caused an escalation of Glu activity on the 45th day of study, in soil contaminated with the highest dose of BPA 800 mg kg⁻¹ dm of soil, while rhamnolipid 90 slightly stimulated biochemical activity soil only for 15 days of the experiment.

The extent of the BPA inhibition as observed in this study corresponded with disorders of spring barley above-ground part growth, and biostimulation augmented the effect. The crop growth stimulation took place only after BPA at the dose of 800 mg kg⁻¹ DM of soil was applied in all the parallel pots, except in those biostimulated with *Chlorella* sp. Nevertheless, the algae were the only ones to significantly reduce the negative BPA impact on the N, Ca and K contents in spring barley.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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