

The assessment of sewage sludge phytotoxicity changes during the processes of composting and vermicomposting

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ABSTRACT

The aim of the research was to evaluate changes of polycyclic aromatic hydrocarbons (PAHs) contaminated sewage sludge phytotoxicity among the composting and vermicomposting processes. The scope of the prepared experiment includes the analysis of *Brassica napus* L. seeds germination and the stem and roots development efficiency. Both analyses were conducted on the incubation baths filled with neutral medium. The prepared research was held in controlled *ex-situ* conditions with the use of four sets of samples—negative control, samples treated with sewage sludge, samples treated with compost, vermicompost use. Each set of samples consist of 9 replicates, 10 seeds for incubation baths. Both compost and vermicompost used during the study were prepared from the same sewage sludge that was included in the set of samples treated only with this material. Based on collected data it can be noted, that composting and vermicomposting showed a positive impact on the removal of organic pollutants such as PAHs from sewage sludge matrix. The implication of this fact has a statistically significant impact on the seeds germination ratio—samples treated with vermicompost showed over 40% higher seeds germination efficiency than samples with raw sewage sludge use. However, at the same time, the additional analysis of plant growth does not show any correlation between PAHs level and the stem and roots growth. It should be also noted that the best environmental results were achieved in the group of samples treated with vermicompost.

Keywords: Sewage sludge; Polycyclic aromatic hydrocarbons; Phytotoxicity; *Brassica napus* L.; Compost; Vermicompost

1. Introduction

The production of sewage sludge (SS) is considered as one of the major problems of modern environmental protection. This material is generated during the wastewater treatment, which classified it as a specific type of organic waste [1,2].

According to the European Council Directive 1999/31/EC, organic wastes with high energy value, such as SS, should be

managed in a sustainable way [3]. This statement originates from the necessity to provide actions aimed at the reduction of carbon dioxide emission to the atmosphere and prevent further environmental damage associated with long term wastes disposal. The importance of mentioned actions comes also from the fact, that a large fraction of organic wastes has high utilitarian potential, that in many cases is untapped. SS, for example, contains a large amount of organic matter, with potentially high energetic and nutritional value. Therefore,

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the disposal of such material may be considered as a waste of economic, environmental, or even social potential [4].

One of the most recommended methods, involved in SS management is the land application. Organic wastes collected from wastewater treatment plants, due to the high nutritional potential can be used directly as fertilizer-like material in agriculture and soil remediation or as a resource for the production of more complex products, such as compost or vermicompost [5]. According to the statistics, this type of management, in 2015 covers 23% of total SS production in Germany, 19% in Poland, and 26% in Lithuania [6]. The most important benefit of the mentioned type of approach is the delivery to the soil of important macro-elements such as nitrogen (N), phosphorus (P), or potassium (K), in the form of complex organic compounds. From the long term perspective, nutrients in that form are more stable and environmentally efficient than simple, clear forms of mentioned elements, that are consisted of mineral fertilizers. It should be also noted, that the introduction of a large amount of organic matter into the soil, may be considered as carbon sequestration, which is a part of strategies aimed to reduce negative aspects associated with climate changes [7].

Despite presented above benefits, the land application of SS can be also associated with some risks. Most importantly the composition of this type of waste is highly dependent on the type and parameters of wastewater subjected to treatment. Different types of technologies, used during those processes also have an impact on the quality and form of SS. One of the most critical aspects of such a phenomenon, is the possibility of presences of hazardous pollutants, within the SS matrix [8].

Described above aspect of SS use is very important, therefore it should be carefully monitored, both by research organizations and international legislation systems. According to law regulations, prepared on the base of European Commission Directive 86/278/EEC, in the European Union, SS subjected to the land application must meet specific quality standards. Mainly those regulations include norms associated with the pollutants content limitations and biological restrictions focused on the possible presences of pathogenic organisms (*Salmonella* sp., eggs of parasites) [9]. Pollutants such as polycyclic aromatic hydrocarbons (PAHs), due to their common presences and confirmed toxic, mutagenic, teratogenic, and cancerogenic activities are widely considered as one of the most important risk factor, involved in the SS management [10]. Compounds from this group are hydrophobic and can easily form bonds with the three-dimensional structures of organic wastes such as SS. After land application, some fraction of pollutants from the described group may have an impact on the local flora and fauna. Those interactions can lead to the positive metabolic transformations of them, which ends in biodegradation [11,12]. However, in many cases, they can also generate a toxic effect and environmental damage. Despite the direct danger associated with the possibility of the integration of PAHs with the local food-chain, the described phenomenon can also inflict economic losses, for example, decreased yield in agriculture [13]. Unfortunately, the current state of legislation, in many countries around the world, does not include specific regulations or restrictions, related to the PAHs presence in SS subjected to land application. The existed acts in

this matter are also very various and therefore hard to compare to each other [9]. The lack of such limitations may contribute to the generation of number of risks, both to the environment and human health. Due to this fact, more detailed studies and eventual future changes in environmental policy should be taken into consideration [4,9].

Taking into account a described above information, it is important to evaluate the scale and mechanisms involved in interactions between PAHs consisted in SS and other organisms from the local ecosystem. This approach can be additionally highlighted by the fact, that the land application of SS is commonly recommended for soil remediation purposes, which also includes phytoremediation technologies. The aim of the paper was to evaluate potential changes of PAHs polluted SS and the products of its management (C, compost; VC, vermicompost) phytotoxicity to one of the strategic energetic plant in the region of Central Europe—*Brassica napus* L.

2. Materials and methods

2.1. Experimental design

The scope of the prepared experiment includes three stages. The first stage was based on preliminary analysis of SS, C, and VC physico-chemical parameters and pollutant content (heavy metals content, PAHs content). The second stage includes analysis of the impact of the selected waste origin materials on the *B. napus* L. seeds germination and the stem and roots development efficiency. During this stage, four sets of experimental groups, divided according to used waste origin soil additive were prepared. Each group contains nine replicates for each type of treatment: control sample (CS), samples treated with SS, samples treated with C and samples treated with VC. CS and SS experimental groups were prepared in January 2016 and the C and VC groups were prepared in June the same year. Experiments were conducted in the same *ex-situ* conditions. The differences of starting time originate from the fact, that both C and VC used during the experiment were prepared from the same set of SS as those used for the SS samples group. All prepared samples were incubated for 45 d within isolated incubation baths. The dimension of the individual bath was 100/100/150 mm (length/width/height). In the final stage, a post-experimental statistical analysis of collected data was done. On the base of this procedure, phytotoxicity factors for *B. napus* L. were calculated.

2.2. Materials

During the realization of the experiment, a neutral incubation medium for plants (medium Murashige and Skoog, MSO) was used. This medium was used both during the *B. napus* L. seeds germination test and the growth and development of stem and roots analysis. The amount of medium used for the individual incubation bath was equal to 200 mL.

As a waste origin soil additive, the three types of materials were used. First, SS was collected from an industrial wastewater treatment plant (food industry—fruit and vegetable processing plant), located in the Silesia region of

Poland. The specificity of wastewater subjected to treatment in this institution ensures a low level of inorganic pollutants such as heavy metals, within generated SS. Second and third materials were C and VC. Both those products were produced from the same set of raw material: SS (80%), green wastes (15%), and organic fraction of municipal wastes (5%). The composting process was held on prisms, located in the area of the Institute of Infrastructure and Environment in Czestochowa (Silesia region of Poland). The environmental conditions during the start of the process were: temperature 15°C–24°C; 75% humidity. Vermicomposting was carried out in mini aerobic bioreactors, with the use of earthworms from the species *E. Andrei* (Sav.), mean body weight of used organisms was 0.53 ± 0.01 g. Conditions used during the vermicomposting were: temperature 17°C–19°C; 65% humidity; 16:8 LD. Earthworms were added to the raw materials mixture in the form of an adult, 40 worms population.

The doses of all listed soil additives were based on the recommendations published in the Polish Ministry of Environmental Protection act on SS (Dz. U. 2015 poz. 227). The used dose was dedicated to the 3 y application and non-agricultural use. This variant was chosen due to obtaining a maximum possible impact on the studied system. In the case of SS, the amount of applied material for one kg of the medium was equal to 118 g. The doses of C and VC were calculated in a way, that allows introducing to medium, the same amount of dry matter as in case of SS application. For C, the dose was equal to 86 g kg^{-1} and for the VC 80 g kg^{-1} .

Plant subjected to study was *B. napus* L. According to Lewandowski and Rymys [14] this plant is considered as one of the strategic energetic plant for the region of Central Europe. One of the main applications of *B. napus* L. seeds is the production of biofuel. Therefore, the growth of this plant can be categorized as non-agricultural production. The implication of this statement is the possibility to realize cultivation on soils with lower quality standards, such as soils subjected to remediation. The number of seeds used for the preparation of the individual incubation bath was equal to 16 seeds, which gives a total number equal to 144 seeds per the type of treatment.

2.3. Physical and chemical analysis

The evaluation of physical and chemical properties of waste origin materials was conducted before the incubation. The samples subjected to study were collected from the randomly chosen places, from the large prisms. Next, obtained material was air-dried and homogenized with use of electric knife homogenizer. Each analysis was conducted in three replications.

All conducted physical and chemical analysis were performed according to the International standardization system ISO and methods published in the literature [15]. Those procedures include a conductometric analysis of pH in water and KCl medium with the use of Mettler Toledo FiveEasy PLUS FP20 pH (Warsaw, Poland), organic matter analysis based on the loss on ignition parameter (LOI), total carbon (C) content—analysis based on PN-EN ISO 17184:2014-08E, total nitrogen (N) content—analysis based on PN-ISO 11261:2002P, total phosphorus (P) content—analysis based on PN-ISO 11263:2002, and heavy metals content

analysis—cadmium (Cd), chrome (Cr), nickel (Ni), and lead (Pb), based on PN-ISO 11047:2001.

2.4. Determination of PAHs content

The analyses of PAHs content, both in waste origin materials and incubation medium, were conducted on Thermo Scientific (Waltham, Massachusetts, United States) HPLC system “SPECTRA System,” equipped with an autosampler, high-pressure pump, and FLD-detector. The initial preparation of samples for the analysis includes solid-liquid extraction with use of acetonitrile (ACN) as a solvent; pre-filtration of extracts on the micro membrane filters $0.25 \mu\text{m}$ and condensation of the obtained medium on the SPE vacuum system. Tubes used for the SPE were filled with C18 silicon dioxide matrix. The separation of individual PAHs was conducted in a reverse phase system, on the column—Restek Pinnacle II PAH $4 \mu\text{m}$. For this purpose, the ACN and methanol (Me) were used as a mobile phase. Separation program, used during the analysis was based on the gradient change of CAN concentration within mobile phase (0 min–10/90 ACN/Me; 18 min 80/20 ACN/Me). In order to identify a PAH compound within the analyzed mixture, the detection technique supported with the external standard was used—Restek US Environmental Protection Agency PAH mix A (16 compounds). The detailed description of this method can be found in earlier publications—Włóka and Smol [16] and Smol et al. [17].

2.5. Phytotoxicity tests

The phytotoxicity of studied materials was asses on the base of the seeds germination test and the analysis of selected plant stem and roots growth. This procedure was conducted within *ex-situ* conditions in large scale climatic chamber. Conditions used during the study were: day-night cycle $16 \text{ h}_{\text{day}}/8 \text{ h}_{\text{night}}$; temperature $T_{\text{day}} = 21^\circ\text{C}$; $T_{\text{night}} = 18^\circ\text{C}$; air humidity = 75%–80%. Both analyses were conducted during the same incubation cycle. The seeds germination was performed on the 10th day of the incubation. This procedure includes a quantitative calculation of seeds that were able to start germination. In the second part of this experiment—the analysis of stem and roots growth was conducted after 45 d of incubation. This procedure includes measurements of the stem and roots length. In order to evaluate the phytotoxicity of studied material, the data collected during the described above experiments were subjected to post-experimental data treatment.

2.6. Data treatment

Data collected during the study were subjected to the post-experimental statistical treatment, with the use of StratSoft STATISTICA and Microsoft Excel software. During this procedure, a variance levels, within individual groups of results were computed. Method used for this purpose, includes a one-way ANOVA test, followed with post-hoc Tukey test. Based on obtained values, evaluation of statistically valid similarities and differences between studied types of treatments were identified.

In order to asses a potential toxic impact of pollutants to *B. napus* L. the Pearson correlation coefficient, between the

content of individual pollutants groups and the seeds germination efficiency and the stem and roots growth intensively were calculated. Based on the obtained values and the identified statistically valid differences or similarities between sample groups treated with different types of waste-origin soil additives, the phytotoxicity levels were established. For this purpose, a classification presented in Table 1 was used. This classification was based on the modified method designed for the evaluation of fertilizers impact on plants.

3. Results

3.1. Experimental design

Results obtained during the preliminary evaluation of physical and chemical parameters of SS, C, and VC are presented in Table 1. The PAHs content in those materials, analyzed before the application to the incubation baths, is illustrated in Fig. 1.

Based on the results presented in Table 2 it can be noted, that raw SS has the lowest pH value and the highest humidity from all analyzed materials. In the case of carbon, nitrogen, and phosphorus contents, C and VC, showed a larger concentration of those elements than SS. The permutable contents of elements from heavy metals group in SS subjected to use in agriculture are consequently 20 mg kg^{-1} for Cd, 500 mg kg^{-1} for Cr, 300 mg kg^{-1} for Ni, and 750 mg kg^{-1} for Pb. After compare of obtained results with mentioned regulation, it is clear, that all analyzed materials meet physical and chemical standards included in actual law associated with SS land application in Poland and European Union.

It is also worth to mention, that values noted for raw SS were on the highest level from all tested materials. The lowest were observed for VC samples. A similar trend applies to the PAHs content. In this case, however, any comparison to law regulation cannot be provided, due to the lack of dedicated regulations for this parameter in Polish law.

According to the analysis of the variance level, all analyzed groups of data for PAHs content, were significantly different from each other. This fact informs, that the use of composting and vermicomposting technologies have a

statistically valid impact on the changes of PAHs content in those materials.

3.2. PAHs content in incubation medium

Another set of data—Fig. 2, presents the PAHs content analyzed in incubation medium samples. The analysis was conducted 1 week after the application of waste-origin soil additives.

The evaluation of PAHs content within incubation mediums, after application of waste origin materials shows, that such procedure may have an impact on the increase of pollutants content in local environment. Based on the data presented in Fig. 2, it can be indicated, that PAHs with lower molecular weight show a higher level of migration into medium than larger compounds. In the case of Naphthalene and three-ring PAHs the 15% of the total content, consisted of used materials was analyzed in the medium after application. For the four and five-ring PAHs this value dropped to 9% and for six-ring to 1%. After the assessment of the statistically valid differences within studied groups of

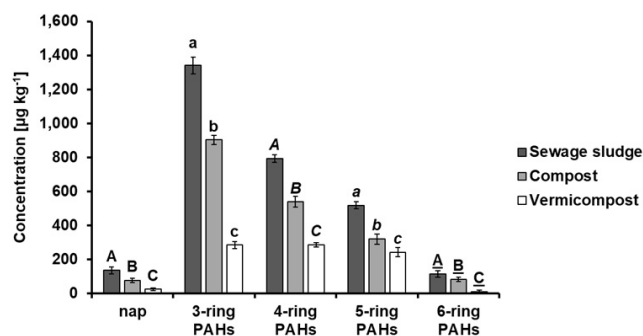


Fig. 1. PAHs content in waste-origin materials. Results presented as means with standard deviation ($n = 9$). Values includes the results obtained during the statistical data treatment—one way ANOVA + post-hoc Tukey tests. Letter and asterisks demonstrate a level of statistically valid similarities or differences between tested types of treatments.

Table 1

List of parameters, used during the evaluation of waste origin materials phytotoxicity

Parameter	Phytotoxicity level			
	High	Medium	Low	Non
Seeds germination (SG)	SG < 40%	SG = 40%–60%	SG = 60%–80%	SG > 80%
	$\rho_{(\sum_{16} \text{PAHs}; \text{SG})} > 0.8$	$\rho_{(\sum_{16} \text{PAHs}; \text{SG})} > 0.8$	$\rho_{(\sum_{16} \text{PAHs}; \text{SG})} > 0.8$	$\rho_{(\sum_{16} \text{PAHs}; \text{SG})} > 0.8$
	ANOVA: $P\text{-value} < F\text{-critical}$	ANOVA: $P\text{-value} < F\text{-critical}$	ANOVA: $P\text{-value} < F\text{-critical}$	ANOVA: $P\text{-value} < F\text{-critical}$
Condition	Or/and	Or/and	Or/and	Or/and
Shoots/roots growth	CS > SS; C; VC (>30%)	CS > SS; C; VC (10–30 > 30%)	CS ≈ SS; C; VC	CS < SS; C; VC
	$\rho_{(\sum_{16} \text{PAHs}; \text{S/R})} > 0.8$	$\rho_{(\sum_{16} \text{PAHs}; \text{S/R})} > 0.8$	$\rho_{(\sum_{16} \text{PAHs}; \text{S/R})} > 0.8$	$\rho_{(\sum_{16} \text{PAHs}; \text{S/R})} > 0.8$
	ANOVA: $P\text{-value} < F\text{-critical}$	ANOVA: $P\text{-value} < F\text{-critical}$	ANOVA: $P\text{-value} < F\text{-critical}$	ANOVA: $P\text{-value} < F\text{-critical}$

SG, seeds germination; S/R, shoots/roots length; ρ , Pearson correlation coefficient; SS, sewage sludge; C, compost; VC, vermicompost.

Table 2
Physical and chemical parameters of sewage sludge, compost, and vermicompost

Parameter	Sewage sludge (SS)	Compost (C)	Vermicompost (VC)
Dry matter [%]	25.1 ± 1.0	32.2 ± 0.8	35.8 ± 0.8
LOI [%]	81.2 ± 0.2	85.1 ± 0.2	84.1 ± 0.4
pH (H ₂ O)	6.59 ± 0.04	7.05 ± 0.02	7.12 ± 0.05
pH (KCl)	6.21 ± 0.2	6.72 ± 0.22	6.69 ± 0.17
C [g kg ⁻¹ _{d.m.}]	259.02 ± 9.0	284.51 ± 12.41	312.08 ± 12.41
N [g kg ⁻¹ _{d.m.}]	5.14 ± 0.45	11.14 ± 1.28	16.32 ± 0.37
P [g kg ⁻¹ _{d.m.}]	1.11 ± 0.12	2.01 ± 0.18	2.91 ± 0.08
Cd [mg kg ⁻¹ _{d.m.}]	0.19 ± 0.03	0.67 ± 0.05	0.76 ± 0.01
Cr [mg kg ⁻¹ _{d.m.}]	15.05 ± 0.12	19.44 ± 0.22	2.15 ± 0.31
Ni [mg kg ⁻¹ _{d.m.}]	0.42 ± 0.05	0.21 ± 0.04	0.08 ± 0.09
Pb [mg kg ⁻¹ _{d.m.}]	5.01 ± 0.17	10.07 ± 0.21	2.07 ± 0.25

LOI, Lost on ignition, results presented as means with standard deviation, $n = 3$.

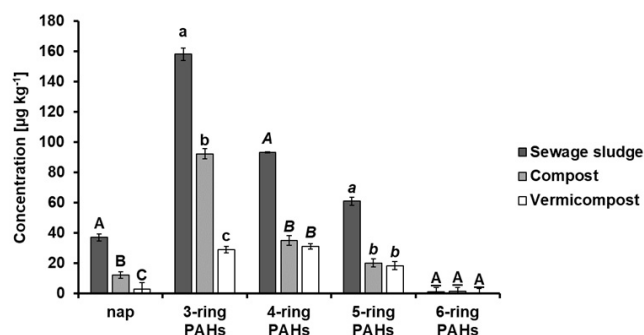


Fig. 2. PAHs content in incubation medium, analyzed 1 week after application of waste-origin materials. Results presented as means with standard deviation ($n = 9$). Values includes results obtained during the statistical data treatment—one way ANOVA + post-hoc Tukey tests. Letter and asterisks demonstrate a level of statistically valid similarities or differences between tested types of treatments.

samples, it should be additionally observed, that the highest differences in terms of pollutants migration ability from the tested material to medium, was noted for naphthalene and three-ring PAHs. In the case of the four and five ringed compounds, the addition of raw SS has the highest variance level, while the effects associated with the use of C and VC were similar to each other. In the case of six-ringed PAHs, all analyzed materials have a similar impact on the change of the pollutant content in the analyzed medium.

3.3. Impact of pollutants content on the growth and development of *B. napus* L.

Data collected during the seeds germination test and the analysis of shoots and roots growth and development are presented in Figs. 3 and 4.

Data obtained during the evaluation of seeds germination efficiency and the effectiveness of shoots and roots growth, allow observing, that the best seeds germination efficiency was observed in control samples and samples treated with VC. Results noted for those groups of samples, according to

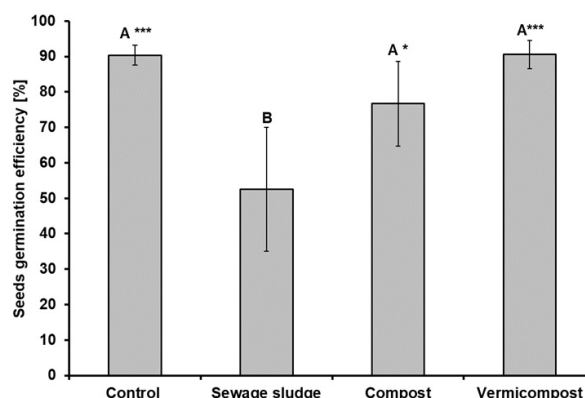


Fig. 3. Seeds germination efficiency, results observed after 10th day of incubation, Results presented as means with standard deviation ($n = 9$). Values includes results obtained during the statistical data treatment—one way ANOVA + post-hoc Tukey tests. Letter and asterisks demonstrate a level of statistically valid similarities or differences between tested types of treatments.

post-hoc Tukey test, were statistically similar to each other. The same analysis shows also, that the use of raw SS and C have a statistically valid impact on the reduction of seeds germination efficiency. The highest loss was observed in case of raw SS use.

The second analysis, focused on the *B. napus* L. growth indicates, that the use of raw SS do not have a statistically valid impact on the shoots development. Results obtained from the samples treated with C and VC, were however higher than those for control samples and samples treated with raw SS. In the case of roots, the addition of waste-origin materials causes a decrease in the length of the studied part of plants. The more detailed evaluation of this parameter, with the use of post-hoc Tukey test, revealed, however, that this effect was not statistically important—all obtained results were statistically similar to each other. Therefore, this parameter should not be taken into consideration during the further assessment of waste-origin materials phytotoxicity.

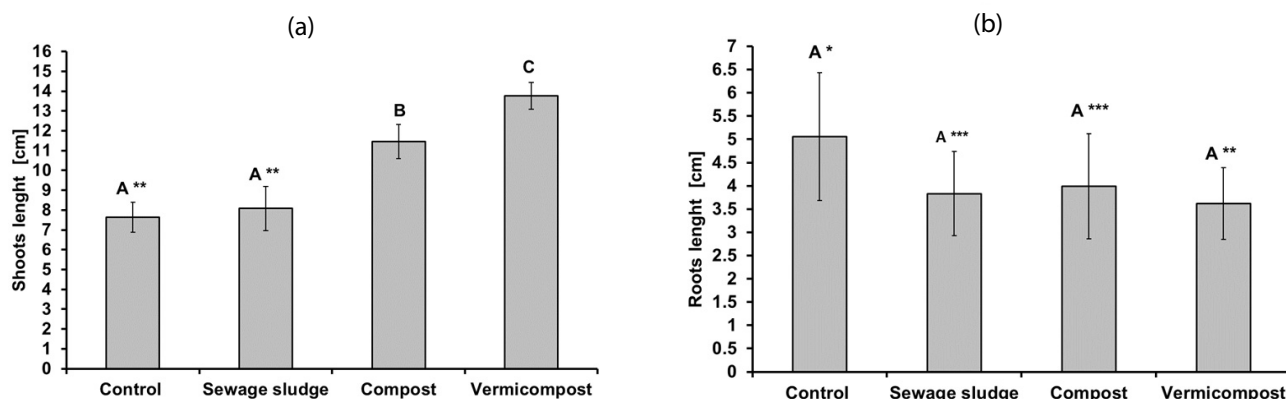


Fig. 4. Shoots (a) and roots (b) growth efficiency, results observed after 45th day of incubation, Results presented as means with standard deviation ($n = 9$). Values includes results obtained during the statistical data treatment—one way ANOVA + post-hoc Tukey tests. Letter and asterisks demonstrate a level of statistically valid similarities or differences between tested types of treatments.

The last set of data consist of results associated with the post-experimental data treatment. The most important coefficients obtained during the execution of this procedure are presented in Table 3.

Based on the data included in Table 3, it should be noted, that all analyzed groups of PAHs showed a negative correlation with the seeds germination efficiency. In the case of shoots and roots growth analysis, the strength of the potential correlation was however on level, that should not be taken into consideration. All analyzed groups of samples have additionally very low value of one-factor ANOVA p -value, which was lower than F -critical. In results this informs, that means within analyzed groups of samples were statistically different to each other. Therefore, observations conducted during the experiment can be subjected to the assessment of the potential phytotoxicity impact.

4. Discussion

4.1. Evaluation of the waste origin materials quality

According to the Polish Ministry of Environmental Protection act on sewage sludge (Dz. U. 2015 poz. 257), all waste-origin materials, used during the study, meet physical and chemical standards required for the application as fertilizer-like material in agriculture (land application) [9,18]. However, based on the other researchers work and due to the approximately high level of PAHs content, noted in studied materials, it should be assumed, that the assessment of

the potential toxicity of waste origin materials to plants was justified. Data presented in Table 2 and Fig. 1 illustrates, that the raw SS has the worst quality from all tested solutions. Such a phenomenon confirms, that the use of additional treatments, like composting or vermicomposting have a positive effect on both the physico-chemical parameters of SS, as well as its level of pollution. The reduction of initial pollutant content may be associated with the initial dilution of substrate. During both implemented processes, the amount of used raw SS was equal to 80% of overall materials subjected to treatment. Such a phenomenon, however, covers only one aspect of the pollutant reduction effect. According to the wide range of literature, managements such as composting and vermicomposting are mostly based on the organic matter transformation which includes mineralization and biodegradation processes. In the case of composting, the main factor responsible for the relevant part of the process consists of various number of aerobic microorganisms. The characteristics involved in metabolic transformations of organic compounds, within such systems, often promotes a pollutant biodegradation process, which leads to the reduction of the initial content of pollutants within the treated medium [19,20]. Vermicomposting, for similar purposes, utilizes more complex biological systems. In this type of method, the mineralization and biotransformation of substrates are carried out by earthworms. This type of organisms are able to consume waste origin, organic materials, and used them as a source of nutrients. During such processes, pollutants accumulated

Table 3

List of parameters, used during the evaluation of waste origin materials phytotoxicity

Parameter	Correlation coefficient						One-way ANOVA	
	Nap	Three-ring PAHs	Four-ring PAHs	Five-ring PAHs	Six-ring PAHs	SUM of 16 PAHs	p -value	F -critical
Seeds Germination	-0.96	-0.93	-0.90	-0.89	-0.91	-0.92	2.177×10^{-8}	2.901
Shoots Length	-0.22	-0.15	-0.01	0.06	-0.22	-0.08	1.591×10^{-17}	2.901
Roots Length	-0.36	-0.39	-0.47	-0.53	-0.34	-0.43	0.036	2.901

SG, seeds germination; S/R, shoots/roots length; ρ , Pearson correlation coefficient; SS, sewage sludge; C, compost; VC, vermicompost.

from the nearest environment are transferred throughout the entire earthworm digestive system, where they are subjected to the interactions with the various enzymatic complexes and symbiotic microorganisms. As in the case of composting, this type of phenomenon can positively affect the biotic degradation of pollutants such as PAHs [21,22].

4.2. Mobility of PAHs within studied systems

The additional analysis of PAHs content within incubation mediums, conducted after the application of waste-origin materials showed, that the use of SS or other materials contaminated with a load of organic pollutants, may generate environmental risk. This issue originates from the fact, that some fraction of pollutants, accumulated within the matrix of organic additive, can migrate to the local environment, which temporally increases a local pollution level. The similar effects were observed both during the realization of earlier studies [23] and after the assessment of other researchers work [24]. The overview of this aspect has been well described in work, published by Oleszczuk [25].

The diversification of specific groups of PAHs, during this analysis, indicates, that such effect is higher for smaller compounds from PAHs group (naphthalene, three-ring PAHs). This phenomenon may result from the properties of low-molecular compounds. According to the scientific data, PAHs with lower number of aromatic rings are more hydrophobic, which makes them more soluble in water. Thanks to that they can be more mobile in environment and also more bioavailable to local fauna and flora [12]. Compounds with larger molecular weight, are more hydrophobic and stable in aquatic systems. In most cases, such compounds are more capable to form bounds with solid particles or elements consisted in organic fraction of soil or other medium. Those properties make them less mobile, which may be considered as explanation of effect observed during current experiment [26,27].

The described effect is very important, especially in cases where soils or other mediums, to which fertilizer-like materials are introduced, are already polluted with the same type of compounds. Many countries around the world do not include specific norms, that regulate the number of organic pollutants, present in materials subjected to land application. The same countries, however, have standards, that cover permitted levels of pollutants in soil used for agriculture or other urban purposes. Such a situation, currently applies to Poland [9,18]. The direct implication of the mentioned case may lead to a situation where the introduction of waste origin soil additive to a specific location, will cause an increase in local PAHs content to level, that will have an impact on the change of the area classification. This type of effect can be considered as a potential source of economic, environmental, or even social problems. For example, this type of issue can inflict delays or even stops in the realization of building projects or other aspects associated with the assumed direction of land use [28,29].

4.3. Phytotoxicity of sewage sludge, compost, and vermicompost

Results presented in Figs. 3 and 4 and Table 3 demonstrate, that the use of waste-origin materials have an impact

on the growth and development of *B. napus L.* Based on the classification included in Table 1, the addition of raw SS into incubation medium have a medium phytotoxic effect. In the case of C, the same data classify it as a low phytotoxic effect. The use of VC however, do not show any statistically valid phytotoxic effect on the studied plant.

The provided classification was mainly based on the results collected from the seeds germination test. This may inform, that pollutants that occurred in studied additives have a higher impact on plants, during the early stages of growth and development. Such effect can originate from the fact, that plant morphology, just after germination, is more volatile to the potential hazardous factors than in older phases of growth. Usually, during this time, plant organisms are not mechanically protected by the specialized tissues, therefore the potential contact between toxic factor and the newly generated plant cells is more probable [30]. Early stages of plant growth, are also associated with the intensification of cell division and DNA multiplication processes. Such time due to the fact, that some pollutants from the PAHs group, can form permanent bounds with the DNA molecules, which may lead to the dangerous errors during genetic information transcription or translation processes, should be considered as a very vulnerable to the induction of mutations and further lethal reactions. The described phenomenon may additionally inflict the decreased seeds germination efficiency, especially in environments where the pollutant content is on a high level [31]. A phenomenon with the high similarity to described in current paper results was also observed by Henner et al. [32].

The data collected from the stem and roots growth analysis, do not meet, assumed in this study, statistical requirements for the phytotoxicity classification. It should be however noted, that the measurements of *B. napus L.* stem growth showed an interesting trend. Despite nutrients provided in the form of organic matter, both control samples and samples treated with raw SS have similar results in the field of stem length. The addition of C and VC, however lead to an increase in the studied plant growth efficiency. This phenomenon may confirm, that the number of pollutants consisted of raw SS, have an inhibitive influence on the development of *B. napus L.* stem. Similar results can be also found in literature. For example, studies conducted by Hamdi et al. [30] and Oleszczuk [10] also showed, that PAHs contaminated soil additives may cause issues in plant growth and development [33]. In the case of roots, all analyzed groups of samples, to which organic materials were introduced, showed a decreased growth of this part of plant. The differences between individual types of treatments were however very diverse, even within the same population. Therefore, those data has not been used during the toxicity evaluation. Despite this fact, a potential explanation of decreased roots length can be associated with the direct delivery of a load of nutrients to the incubation medium. It is possible, that plants in those samples have better access to the micro and macro elements, which may inhibit the growth of underground organs of plants [34].

The described above the toxic and semi-toxic effect of waste-origin materials to *B. napus L.* informs that such type of additives subjected to the land application should be monitored in terms of the eventual level of pollution with

the compounds from PAHs group. The lack of centralized law regulation, in this matter, combined with an observed phenomenon, may contribute to the generation of a number of economic, social, environmental, and even health risks. Therefore, it is important to take actions aimed at the development or novelization of actually existed legislation acts. Such a procedure is very critical, and should be carried out with the regards to the necessity to preserve a sustainable development and circular economy principles. To high restrictions in this field can have a negative impact on the implementation of the mentioned strategies in practice. The lack or to the liberal character of such action, from the other point of view, can be not sufficient for the environmental and human safety requirements. Based on this statement it is clear that all further research, conducted within similar field as the current study, may provide more impartment data, that will be useful for both practical and organizational purposes [4,35].

The necessity to conduct research on a larger scale, within the described field, can be also highlighted by the fact, that the characteristics and form of waste-origin materials are highly diverse. Aspects such as type of wastes (substrates), method of management, type of post-processing treatment, etc. have a confirmed impact on the final form and composition of the end product. Currently, available technologies involved in wastewater treatment, composting and vermicomposting have also different characteristics. In the last few years, the mentioned phenomenon, due to the high focus on the research and development sector has grown to the scale, that is difficult to summarize in one actual existing law regulation. Therefore, it is important to preserve within newly created legislation, a dedicated space for the implementation of new innovative methods and technologies, that can solve key aspects of studied issues, without additional negative impact on economy, environment, or social communities [4,36,37].

5. Conclusions

In conclusion, results presented in current study informs, that the use of PAHs polluted organic soil additives, such as sewage sludge or products of its management, despite the fact, that they meet quality standards included in the law for land application, may have a negative impact on the *B. napus L.* growth and development. Additionally, data collected from the same experiment allow us to observe, that composting and vermicomposting processes, through the removal of PAHs from the substrate matrix, can reduce a toxic effect to studied plant. The mentioned effect was statistically valid for the seeds germination tests. It may inform, that early stages of *B. napus L.* growth were more volatile to potential toxic interactions. Data obtained for stem and roots growth analysis was not sufficient to provide statistically valid observations in the current study, therefore the further research in this matter should be performed.

The statistically valid observations based on the *B. napus L.* growth indicates, that the lack of specific law regulation on the organic pollutant limits in materials subjected to the land application may be considered as an environmental, social, and economical risk factor. Therefore, it is important to conduct more detailed research within the described field and utilize obtained data in new law regulations.

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