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Relevance of Pyrolysis Products Derived from Sewage Sludge for Soil Applications

Vladimír Frišták [1,*], Diana Bošanská [1], Vladimír Turčan [1], Martin Pipíška [1], Christoph Pfeifer [2] and Gerhard Soja [2,3]

[1] Department of Chemistry, Trnava University in Trnava, 91843 Trnava, Slovakia
[2] Institute for Chemical and Energy Engineering, University of Natural Resources and Life Sciences Vienna, 1190 Vienna, Austria
[3] Energy Department, Austrian Institute of Technology GmbH, 3430 Tulln, Austria
* Correspondence: vladimir.fristak@truni.sk or fristak.vladimir.jr@gmail.com; Tel.: +421-33-592-1459

Abstract: The recovery of sludge produced in the wastewater treatment process in WWTPs is often limited by the high content of toxic forms of contaminants of both an inorganic and organic nature. One of the options for the effective treatment of the world's ever-increasing quantities of sewage sludge is the pyrolysis process. Thermochemical conversion of sewage sludge is emerging as a promising method for treating these heterogeneous and highly complex wastes with increasing research work. Pyrolysis-treated sewage sludge (PM) prepared at 603–615 °C was characterized by pH, EC, and CHN-S analysis; total and bioavailable concentrations of P and heavy metals (Cd, Cu, Fe and Zn); fractionation of bound forms of P and heavy metals in the material and determination of the presence of polycyclic aromatic hydrocarbons (PAHs). The studied material was subjected to ecotoxicological tests (*Daphnia pulex* L.) and cultivation tests (*Lactuca sativa* L.). Elemental analysis revealed the concentrations of heavy metals in PM: Fe (137,600 mg/kg), Zn (2602 mg/kg), Cu (582 mg/kg), Cr (107 mg/kg), Pb (87 mg/kg), Ni (67 mg/kg), As (<1 mg/kg), Hg (<2 mg/kg) and Cd (<1 mg/kg). The highest values of extractability of the investigated heavy metals from PM were found in the cases of Zn (HCl) and Fe (Mehlich 3), both values not exceeding 500 mg/kg. BCR sequential extraction showed the major concentrations of Cu and Fe were predominantly bound in the residual fraction (F4) and Zn in the reducible fraction (F2) of PM. The results of heavy metal bioavailability suggest that the addition of PM does not negatively affect the growth of lettuce biomass and the metal contents of plant tissues. Based on the results obtained, the pyrolysis material prepared from municipal sewage sludge seems to be a promising and innovative soil additive and a potential alternative to conventional inorganic fertilizers.

Keywords: sewage sludge; pyrolysis; soil amendment; fertilizer; heavy metals; bioavailability

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1. Introduction

The global population is estimated to reach more than 9 billion by 2050, multiplying the demand for electricity, water and essential food [1]. Similarly, waste generation will grow in proportion to population growth as well as consumption rates. There arises the question of how to meet all these requirements without absolutely depleting natural resources [2]. Waste is very often an undervalued raw material that can be effectively used alongside conventional incineration or landfilling [3]. Within the EU, several legislative steps have been implemented in the last 20 years that should lead to the reduction and especially to the recycling of the waste produced, with an ultimate positive impact on the environment. The European Commission considers that, if waste can be used as a source of raw materials, more activity is needed and, in particular, greater priority should be given to waste treatment technologies that lead to its re-use and recycling [4,5]. Organic waste accounts for approximately 46% of the total amount of solid waste produced. In

addition to the traditional landfill method, new or modified technologies and processes can be used for their treatment, leading to progressive and innovative products [6–8]. The use of unprocessed organic waste in agriculture can bring with it a number of benefits, such as improved soil structure, pH adjustment, an increase in nutritionally important elements and, last but not least, a reduction in the use of inorganic forms of fertilizers. On the other hand, there are a number of negatives, such as pollution of surface and groundwater with nitrogen, phosphorus and pathogens, emissions of ammonia, nitrogen oxides, methane and the accumulation of phosphorus, zinc, copper and sodium in the soil [9]. However, organic wastes represent a huge source of organic matter, and with proper technological treatment, their ecological, agronomic and economic value can be enhanced. One of the important environmental questions we are asking ourselves is about the disposal and treatment of sewage sludge [10–12]. The production of bulk sewage sludge is growing at an enormous rate with the expansion of human society and global industrial activity [13]. Any use of sewage sludge is subject to strict legislative measures due to its content of a wide range of pollutants, including heavy metals. In the case of sewage sludge application, heavy metals can negatively affect soil organisms due to inhibition of metabolism, low biomass productivity, impact on reproduction and, in extreme cases, mortality of the individual [14]. On the other hand, sewage sludge is considered to be an important source of nutritionally important elements (N, P and Si) and essential elements (Zn, Fe, Cu and Ni), which are required in certain quantities by soil organisms and plants [15]. In recent years, thermochemical methods such as hydrothermal carbonization, incineration, gasification and pyrolysis have gained popularity for the recovery of sewage sludge [16]. In the case of pyrolysis, it is the thermochemical conversion of the carbonaceous fraction of sludge into a more stable product rich in nutritionally important elements, whose application in soil applications as additives in agriculture, horticulture and ornamental beds as well as substrates for so-called green roofs represents a vision for the treatment of this heterogeneous waste material [17–19]. The solid pyrolysis product is generally characterized as a microporous material with a large specific surface area, abundant oxygen-rich functional groups and a relatively high pH and cation exchange capacity (CEC) [20]. These properties are believed to have a great contribution to stabilize the forms of heavy metals present and reduce the concentration of microbial pathogens and organic contaminants [21]. Therefore, it is necessary to promote scientific ideas that are directed towards basic and applied research devoted to the application of traditional technologies in relatively new sludge treatment applications. Although pyrolyzed sludge appears to be a potential fertilizer, EU legislative regulations do not yet allow the use of this material in agriculture, so future scientific studies should focus on investigating the ecotoxicity and bioavailability of pollutants mobilizable from pyrolyzed sludge.

Based on these scenarios, the main research objective of this study was to investigate the main characteristics of pyrolysis material prepared from municipal sewage sludge as a potential soil amendment. We attempted to evaluate the pyrolysis material produced in terms of the content and accessibility of nutritionally important elements as well as potentially toxic elements.

2. Materials and Methods

2.1. Pyrolysis Material

Pyrolysis material based on sewage sludge (PM) was produced in cooperation with the administration of the Linz am Rhein municipal association as a test product within the Linz-Unkel WWTP (Linz, Germany). A large-capacity Pyreg continuous pyrolysis reactor with a pyrolysis temperature of 603–615 °C, a strictly anoxic atmosphere and direct combustion of the resulting gases (FLOX-plant) was used for the production. The obtained pyrolysis material was sieved to achieve a fraction of 0.5–1 mm.

2.2. Physico-Chemical Characterisation of the Material

The active pH was determined by adding 0.5 g of PM sample to 7.5 mL of deionized water to maintain a 1:15 *w*/*v* ratio, and the mixture was then stirred for 1 h at 45 rpm on a laboratory shaker (Orbital Shaker Multi-RS 60, Biosan, Latvia). To determine the potential pH, 7.5 mL of KCl at a concentration of 1 mol/L was added to 0.5 g of PM sample and allowed to stir similarly for 1 h at 45 rpm on a laboratory shaker. In both cases, the suspensions were allowed to stabilize for 1 h after stirring, and then the active and potential pH values were measured with a pH meter (pH/EC Multimeter 3420, WTW, Germany). The measurements were performed in triplicate each time. The conductivity was determined by adding 7.5 mL of deionized water to 0.5 g of PM sample. Conductivity was then measured in the supernatant (pH/EC Multimeter 3420, WTW, Germany). The ash content was determined by the method of Rehrah et al. [22]. Carbonate content was determined in PM samples by the volumetric method with the application of Janko's calorimeter. The total C, H and N contents of the sample were quantified by an elemental analyzer (CHNS-O EA 1108, Carlo Erba Instruments, Milan, Italy). Total metals concentrations in PM were determined by ICP-MS (Perkin Elmer, Elan DRCe 9000, Shelton, CT, USA) and RFS (Spectro-Xepos, Kleve, Germany). To determine the total concentration of 18 structures of polycyclic aromatic hydrocarbons (PAHs) according to USEPA (1995): naphthalene, acenaphthylene, acenaphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, ideno(1,2,3)pyrene, 1-methylnaphthalene and 2-methylnaphthalene, a modified extraction procedure according to Hilber et al. [23]. Briefly, we weighed 5 g of PM sample into the extraction cartridges. The cartridges were placed in a Soxhlet extraction apparatus and 125 mL of extraction reagent, toluene (p.a), was added to the boiling flask. The boiling flask was heated at <115 °C to achieve boiling of the extraction reagent. The extraction was carried out for 36 h. The obtained extracts were concentrated using a Hei-VAP vacuum evaporator (Heidolph Instruments GmbH, Schwabach, Germany) at conditions of 42 °C, 140 rpm and 50 mbar of pressure. High performance liquid chromatography 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) was used for the quantification of PAH compounds using a Restek C18 PAH column (150 × 4.6 mm; 4 µm), acetonitrile-water mobile phase and a DAD detector (λ = 280 nm). Sample volume was 5 µL and the temperature was 30 °C. Inorganic forms of phosphorus in the biochar sample were detected by acid extraction using 1 mol/L HCl. By subtracting the inorganic P values from the total P, we calculated the proportion of organic P in the PM sample. Total phosphorus was determined UV-VIS spectrophotometrically after prior wet oxidation with HNO_3 and H_2O_2 [24]. The phosphorus binding in the PM was determined by sequential extraction. In three repetitions, dried material samples (0.5 g) were sequentially extracted in the order of 25 mL distilled water, 0.5 mol/L $NaHCO_3$, 0.1 mol/L NaOH and 1 mol/L HCl. The readily soluble forms of phosphorus and its calcium compounds were detected by the calcium–acetate–lactate (CAL) method. To assess the mobility of bound heavy metals (Cd, Cu, Fe and Zn) in the pyrolyzed PM sample, we performed a series of one-step extraction protocols (deionized water, DTPA, 0.1 mol/L HCl and Mehlich 3 extraction mixture). The fractionation of bound metals was monitored using a BCR sequential extraction protocol. The quantification of heavy metals in the PM matrix as well as in the extracts obtained in the one-step and sequential protocols was carried out by F-AAS method using Agilent 240FS AA (Agilent Technologies, Santa Clara, CA, USA).

2.3. Ecotoxicological Tests

An acute toxicity test using the common daphnia (*Daphnia pulex* L.) was used to monitor the effect of the investigated pyrolysis material on aquatic fauna. Test specimens were obtained from commercially available farms, stored in a refrigerator at 5 °C, and applied to the test immediately after collection. The age of larvae was estimated to be 3–4 days based on morphological characters. The test medium was enriched with 6.25%, 12.5%, 25%, 50% and 100% aqueous extract of PM (2 h, *w*/*w* deionized water/PM, 2/1),

inoculated with daphnia larvae (five individuals) and exposed for 48 h, at 25 °C, and a light regime of 16 h/8 h (light/dark).

The bioavailability of heavy metals in the pyrolyzed sludge was also investigated by a culture test using a plant model of lettuce (*Lactuca sativa* L.). The lettuce seeds were allowed to germinate on chemically clean sand in crystallization trays. Subsequently, we cleaned the sprouts and transferred them into clean plastic containers filled with garden substrate, with the addition of PM 5%, 10% and 20% (*v*/*v*). The experiments were carried out in three replicates with a control sample consisting of pure commercial garden substrate and a sample with 100% PM. The samples were moistened with deionized water to reach 60% soil moisture, which was maintained in the containers for 4 months while the samples were placed indoors in the laboratory (mode 10 h/14 h light/dark, 22 °C). Subsequently, the obtained lettuce biomass samples were washed in deionized water to remove residual substrate and dried in a laboratory oven at 60 °C. The homogenized samples were subjected to wet digestion at 180 °C using digestion digesters and concentrated HNO_3 as a mineralizing agent. The obtained digests were filtered through a syringe filter with a PTFE membrane (0.45 µm) and stored in a refrigerator at 5 °C. For the quantification of metals in digests of plant materials, the ET-AAS method using Agilent 240FS AA (Agilent Technologies, Santa Clara, CA, USA) was used.

3. Results

The basic physicochemical characterization of the pyrolyzed material (PM) produced from municipal sewage sludge showed the active pH of the material to be in the neutral region (Table 1). In their work, Zielińska et al. [25] dealt with the pyrolysis of sewage sludge at temperatures of 500 °C, 600 °C and 700 °C, and they observed an increasing trend of active pH values with increasing pyrolysis temperature. They justified this process by the fact that during the thermal treatment of the sludge, polymerization/condensation reactions of aliphatic compounds occur, which are minimal at low temperatures, and at the same time the amount of acidic functional groups on the surface of the pyrolyzed sludge is reduced. The same trend was described by Wang et al. [26], who investigated the pyrolysis of sewage sludge at temperatures of 350 °C, 550 °C and 750 °C and observed an increase in pH values towards the alkaline region. In the case of our PM sample, the potential pH value was in the weakly acidic region (pH < 7). In their work, Mierzwa-Hersztek et al. [27] reported neutral values of potential pH of the material obtained by pyrolysis of sewage sludge from different regions of Poland (Krakow, 6.89; Krzeszowice, 7.06; Slomniki, 7.18) at 300 °C. In contrast, de Souza et al. [28] reported an increasing trend in potential pH due to pyrolysis temperature (300 °C < 500 °C < 700 °C), attributing this change, as mentioned above, to the loss of acidic functional groups (carboxyl, hydroxyl or formyl) from the surface of the pyrolyzed sewage sludge. The increase in the alkaline character of the pyrolyzed sludge may be due to the separation of alkaline elements (Ca, Mg and K) from organic compounds during the pyrolysis process. The increase in alkaline character of pyrolyzed sewage sludge can be justified based on the ongoing polymerization/condensation reactions of aliphatic compounds; dehydration of the sludge, which leads to a reduction in the amount of acidic functional groups; and last but not least, the removal of alkali metal salts from the organic matrix with increasing pyrolysis temperature [29]. At a higher pyrolysis temperature, the alkali salts in the clarified sludge are released from the structure and the oxygen functional groups are decomposed, leading to a reduction in the number of acid functional groups. The pH value of the pyrolyzed sludge is also significantly affected by its increased aromaticity of material structure [30].

Table 1. Physico-chemical characteristics of pyrolyzed material (PM.).

pH_{H2O}	7.03 ± 0.03
pH_{KCl}	6.58 ± 0.04
EC * (µS/cm)	1381.67 ± 109.56
Ash content (%)	0.09
CEC ** (meq/100g)	19.47 ± 6.59
$CaCO_3$ (%)	0.2 ± 0.01
C (%)	35.5
H (%)	0.95
N (%)	4.21
As (mg/kg)	<LOQ ***
Cd (mg/kg)	<LOQ ***
Cr (mg/kg)	107
Cu (mg/kg)	582
Ni (mg/kg)	67
P (mg/kg)	60,230
Pb (mg/kg)	87
Zn (mg/kg)	2602
Fe (mg/kg)	137,600
Hg (mg/kg)	<LOQ ***
16 US-EPA PAHs (mg/kg)	1.054

* EC—electrical conductivity; ** CEC—cation exchange capacity; *** Limit of quantification (LOQ); RFS: As 1 mg/kg, Cd 1 mg/kg, Hg 2 mg/kg.

The resulting electrical conductivity (EC) value of PM is greatly influenced by the composition of the feedstock, the pyrolysis conditions (time and temperature) and the treatments prior to pyrolysis. Compared to other works that dealt with the pyrolysis of sludge at approximately the same temperature (600 °C), the EC value is more than 3-fold lower [31]. Authors also noted in their work that as the pyrolysis temperature increases (260 °C < 420 °C < 610 °C), the EC values of the pyrolyzed sludge also increase. The increase in EC values is due to the accumulation of chemical compounds containing Na, K, Ca and Mg. During increasing pyrolysis temperature of the sewage sludge, the solubility of salts and metals decreases, while at temperatures > 500 °C, these compounds are incorporated into Si-containing structures, forming insoluble salts [28]. EC values are among the important parameters in the application of PM to alkaline soils (most dry soils), which affect soil salinity and excess Na^+ ions in the soil [31]. Racek et al. [14] state that the CEC value represents indicators of exchangeable cations such as Ca^{2+}, Mg^{2+}, K^+ and Na^+, which are important indicators of soil quality and productivity. CEC value of PM 19.47 ± 6.59 meq/100 g of pyrolyzed material was comparable to published CEC values of materials produced at the same temperature from sewage sludge. In the work of de Souza et al. [28], the authors reported that PM prepared at pyrolysis temperatures up to 480 °C tends to have higher CEC values because some oxygen functional groups, such as phenolic and carboxyl groups, are retained, and conversely, PM processed at temperatures above 480 °C has lower CEC values. Although the CEC values of the pyrolyzed material vary considerably between the treatment sludges, in general, as the pyrolysis temperature increases (>300 °C), the CEC values decrease. This may be due to the reduction of functional groups and oxidation of aromatic functional groups by temperature, usually characterized by a decrease in the O/C ratio with increasing pyrolysis temperature [32]. For the elemental analysis of PM, we focused on the total contents of C, H, N, P and selected heavy metals, such as As, Cd, Cr, Cu, Ni, Pb, Zn, Fe and Hg according to EBC [33]. The total concentrations of C, H and N were consistent with those values found in pyrogenic carbonaceous materials produced from municipal sewage sludge by several authors [15,17,25]. In general, the total content of C in pyrolyzed sludge is reduced compared to native sludge feedstock. This is due to the degradation and volatilization of C-rich organic compounds that cannot be converted into stable C-forms. Additionally higher content of N is dependent on higher concentrations of N-rich substances in raw sludge. The concentrations of the heavy metals

studied decreased as follows: Fe > Zn > Cu > Cr > Pb > Ni > Hg > As, Cd. The highest abundance in PM was observed in the case of Fe, whose values exceeded by more than 52 times the values of the other heavy metals studied. Zoghlami et al. [31] reported an increase in Fe concentration in PM with increasing pyrolysis temperatures. This finding supports the fact of the decomposition of organic compounds, the increase of solids content in PM and the multiplication of element concentrations. The values of Fe concentrations in PM were almost 3-fold lower compared to the concentrations found in our PM sample. Zhang et al. [30] reported an increasing trend of Zn concentration in PM with increasing pyrolysis temperature in their work, and the values reported in their work were more than 9-fold lower compared to the Zn concentration in our material. This trend was equally noted in the work of Mierzwa-Hersztek et al. [27], who reported Zn concentrations that were 2-fold lower compared to our PM sample. The higher concentrations of Fe and Zn in the pyrolyzed sludge are due to the higher concentrations of these elements in the input raw treatment sludge. The increased concentrations of heavy metals in PM were probably due to the pyrolysis temperature affecting the dissociation of organic compounds and some minerals, such as carbonates. For example, chlorides and sulfide-chloride compounds of heavy metals in scrub sludge are easily sublimable, but sulfide compounds of heavy metals are more resistant to this process [27]. On the other hand, the lowest abundance in PK was observed for As, Cd and Hg, whose concentrations were less than < 2 mg/kg. We assumed very low values of Hg concentration in PM because Hg compounds are volatile even at pyrolysis temperatures lower than 600 °C. The decrease in Cd concentrations can be attributed to the reduced formation of oxide in the reducing atmosphere during the pyrolysis process, which contributes to the formation of volatile forms of Cd. A decreasing trend in Cd concentration was also noted by Zoghlami et al. [31] and Mierzwa-Hersztek et al. [27]. The CAL extractable forms of phosphorus in the sample, which as chemical tools characterize the amount of bioavailable phosphorus to plants, contained a concentration of 7.25 mg/g, which is approximately 12% of total phosphorus. From the P-sequential extraction (Figure 1), we determined the abundance of phosphorus forms present in PM. Soluble inorganic orthophosphates, which are readily available to plants as a rapid source of phosphorus, are extractable using H_2O and $NaHCO_3$ as extraction agents [34]. Based on our results, we can conclude that the mobile water-extractable forms of P were relatively poorly represented in PM, which is due to the pyrolysis treatment of the sludge that transformed phosphorus into more stable forms in the pyrolysis product. NaOH-extractable phosphorus represents orthophosphate composites with Fe and Al, which are slowly released into soils [34] and, in the case of PM, represent a relatively high value (>27%). HCl-extractable phosphorus represents forms of more stable phosphorus and minerals that are released very slowly into the soil, and the value of HCl-extractable phosphorus was represented in the highest proportion (>34%) in our pyrolysis sample. Non-extractable P, as a residual that is not mobilizable by any of the extraction reagents used, accounted for 40%.

The total polycyclic aromatic hydrocarbon content specified as the sum of extractable concentrations of 16 PAH structures according to US-EPA [35] from PM was 1.054 mg/kg (Table 1), which is lower compared to the requirements of EBC (2012), which limits the concentration of PAHs to 12 mg/kg for conventional pyrolysis materials or 4 mg/kg for premium pyrolysis materials. The predominant PAHs present were hydrocarbons with 4-ring structures, followed by 3-ring structures. Due to the pyrolysis material preparation temperature, cracking of these structures into 2- and 3-ring hydrocarbons may occur; however, according to Tomczyk et al. [36], the formation of multi-ring hydrocarbons occurs with increasing temperature. Additional analysis of the methylated hydrocarbons only revealed the presence of 2-methylnaphthalene at a concentration of 0.129 mg/kg, which according to Frišták et al. [17] has greater volatility than the other PAH substances, making it more hazardous. The presence of PAHs in the sample of PM demonstrates the potential risk of contamination and intoxication when applied to soil. However, the

determined concentrations represent so-called total concentrations and are not readily mobile or mobilizable under normal environmental conditions.

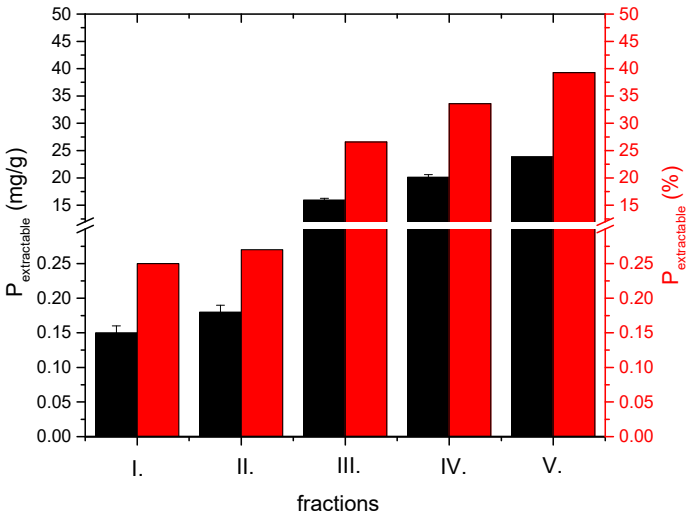


Figure 1. Fractionation of bound phosphorus in pyrolyzed material (PM) determined by sequential extraction with the following order of steps: H_2O-extractable P (I), $NaHCO_3$-extractable P (II), NaOH-extractable P (III), HCl-extractable P (IV), and residual (non-extractable) P (V).

To investigate the bioavailability of heavy metals from PM, we focused on Cd, Zn, Cu and Fe using one-step extraction protocols using multiple extraction agents—deionized water (DW), DTPA, HCl and Mehlich 3 (Figure 2).

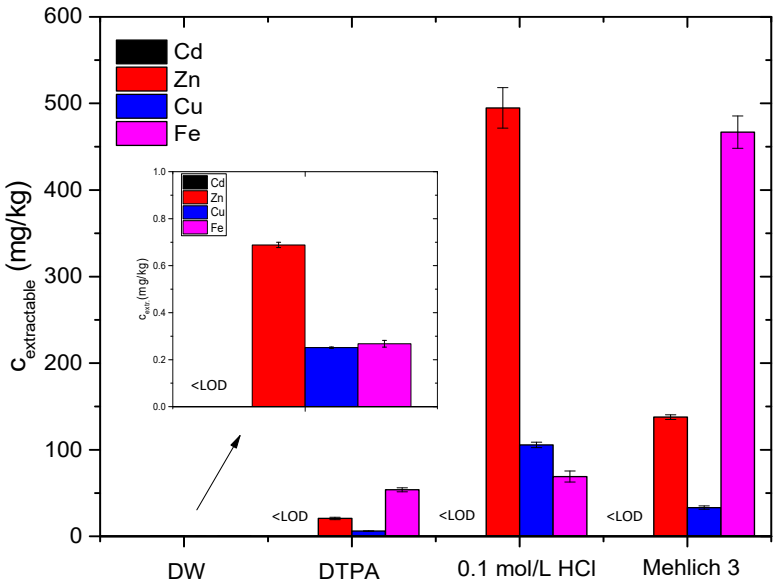


Figure 2. Extractability of Cd, Zn, Cu and Fe (mg/kg) from PM in the one-step protocols using deionized water (DW), diethylenetriaminepentaacetic acid (DTPA), 0.1 mol/L HCl and Mehlich 3 extraction reagents. The values of extractable Cd under the given conditions were lower than the limit of detection for the given concentration determined by the F-AAS method (<limit of detection-LOD).

In the case of Cd and its extractability, values were recorded that were lower than the detection limits of the analytical method used. By using DW as an extraction agent, we were able to obtain the lowest values of extractable concentrations of the studied heavy metals from PM compared to the other agents. The concentrations of the investigated heavy metals did not exceed < 1 mg/kg, while the highest values of extractable forms were observed in the case of Zn (0.7 mg/kg). The concentrations of heavy metals obtained using

DW decreased in the order: Zn > Fe > Cu > Cd. The results show that the accessibility of water-soluble heavy metals is minimal, and such forms of heavy metals do not pose a significant risk to soil ecosystems. In the work of Barry et al. [37], the authors addressed the characterization of PM obtained by fast and slow pyrolysis (300 °C–500 °C) of sewage sludge and found that higher concentrations of heavy metals extracted by this extraction reagent were dependent on the pyrolysis rate, higher ash content and reduced total C in PM when extracted by DW. In our case, we observed a very low value of ash content in PM in the physicochemical analysis (Table 1), from which a low extraction efficiency by DW could be assumed. Using the chelating agent DTPA, we extracted higher concentrations of heavy metals from PM compared to the values obtained from extraction by DW. The extractable concentrations of heavy metals from PM did not exceed the values of 60 mg/kg and the highest values were observed in the case of Fe. The extractability of heavy metals from PM using DTPA decreased in the order: Fe > Zn > Cu > Cd. Lu et al. [38] investigated the leachability of heavy metals (Pb, Zn, Cu, Cd, Fe and Mn) from pyrolysis treated sludges from different areas obtained at temperatures between 300 and 500 and observed an increasing trend of extracted Pb, Zn and Cu with raised pyrolysis temperature. The highest extractable concentrations of Fe were recorded for materials produced at a pyrolysis temperature of 400 °C in all the investigated PMs. Nitrogen-containing functional groups such as amine-amide groups that are conserved at 300 °C can act as ligands in the sludge, binding with the metals and incorporating into the rest of the carbon structures. Similarly, functional groups (carboxyl and hydroxyl) on the sludge surface at lower pyrolysis temperatures contribute to the formation of organometallic complexes in PM structures. In the work of Lu et al. [39], the authors reported that the contents of bioavailable Pb, Cd, Ni and Cr in PM prepared at different pyrolysis temperatures (300 °C–700 °C) were similar. In conclusion, this work hypothesizes that pyrolysis temperature does not have a consistent effect on the bioavailable heavy metal contents of PM, but the pyrolysis process can suppress the release of heavy metals extractable by the DTPA reagent by forming organometallic complexes. For the bioavailability of heavy metals extractable with HCl from PM, we found the highest concentrations in the case of Zn quantification. The value of the extractable Zn concentration (500 mg/kg) obtained using HCl was the highest value among all the single-step extraction protocols used and the metals determined. The values of heavy metal concentrations in PM subsequently decreased in the direction Zn >> Cu > Fe >> Cd. The values of Cu and Fe concentrations in PM were 5-fold lower compared to the value of Zn concentration. The one-step extraction using Mehlich 3 extraction agent (M3) models the conditions for a weakly acidic soil environment. The highest value of M3 extractable concentration from PM was recorded for Fe, while the values of the selected heavy metals investigated decreased in the direction: Fe >> Zn > Cu >> Cd. The values of Zn and Cu concentrations in PM extractable by Mehlich 3 agent were 3-fold lower compared to the value of Fe concentration. The obtained results suggest that the potential application of PM to more acidic soils could cause a significant release of Fe and Zn containing compounds. The high concentrations of extractable forms of Zn and Fe corresponded with the high values of total Zn and Fe contents in PM obtained by elemental analysis (Table 1).

The BCR sequential extraction protocol was used to quantify the individual fractions of Zn, Cu and Fe in pyrolyzed sludge (Figure 3). Sequential extraction showed that Zn occurs in PM bound predominantly in the reducible fraction (F2) and accounts for approximately 46% of the total Zn concentration; further, 34% of Zn is bound in the poorly available residual fraction (F4) and 19% of Zn is bound in the exchangeable and acid-soluble fraction (F1). A very small percentage (<1%) of the extracted Zn concentration occurs bound in the oxidizable fraction (F3) of the PM. Lu et al. [38] reported relatively high percentages of Zn in the F3 and F4 fractions of PM, with both studies noting an increase in the residual Zn fraction with increasing temperature during the pyrolysis of the sewage sludge. In the case of Cu concentrations, the predominant concentration is bound in the residual fraction—50% (F4) and the oxidizable fraction—40% (F3). Lower Cu concentrations were observed bound in the reducible fraction—9% (F2), and the exchangeable and acid-soluble

fraction—1% (F1). Liu et al. [40] reported similar values for the percentage concentrations of Zn and Cu in PM obtained from sewage sludge by pyrolysis at 650 °C. The bulk of the percentage concentration of Fe in PM was in the residual fraction—79% (F4), and the reducible fraction—21% (F2), while the remaining fractions (F1, F3) were not present in PM. Alipour et al. [41] reported that as the pyrolysis temperature of sewage sludge increases, the concentrations of heavy metals bound to the immobile fractions (F3 and F4) also increase compared to the mobile fractions (F1 and F2), thus decreasing the potential accessibility and risk of ecotoxicity of heavy metals present in the original sludge. In their study, Zhang et al. [30] reported that the bioaccessible concentration is characterized by just the exchangeable and acid-soluble fraction (F1) and the reducible (F2) fraction of heavy metals, which are highly susceptible to leaching and mobilization, respectively. The potentially bioavailable concentration is related to the oxidizable (F3) fraction, which is subject to leaching and degradation but only under extreme conditions (a strong oxidizing environment and extremely acidic conditions at pH $\leq$ 3). The residual fraction (F4) of heavy metals is not susceptible to degradation and leaching and is therefore not classified as a bioavailable fraction. From the obtained results, it is evident that Cu contained in raw sewage sludge is transformed by the pyrolysis process into organic PM structures, which may cause potential ecotoxicity after application to the soil and may be bioavailable to the soil ecosystem, based on soil properties (pH, nutrient composition, etc.). Results from single-protocol extractions of Zn and Cu from PM confirmed that Zn was primarily present in the bioavailable F1 and F2 fractions of PM. Although from the BCR sequence analysis of PM we found that Fe was predominantly present in the residual fraction (F4), we measured relatively high concentrations of leachable Fe using the Mehlich 3 reagent when determining the bioavailability of heavy metals in PM. In their study, Zhang et al. [30] concluded that the bioavailable concentrations of heavy metals (F1, F2) are transformed into stabilized components (F3, F4) with increasing pyrolysis temperatures. This transfer of heavy metals is due to the fact that the organic matter present in the purified sludge contains diverse complex functional groups (-COOH, -OH and others) and chelating groups (-NH_2, -SH and others). During the pyrolysis process, the organic material is decomposed, the number of functional groups on the surface of the sewage sludge is reduced and the heavy metals are transformed into stable sulfides or oxides, leading to a reduction in their mobility.

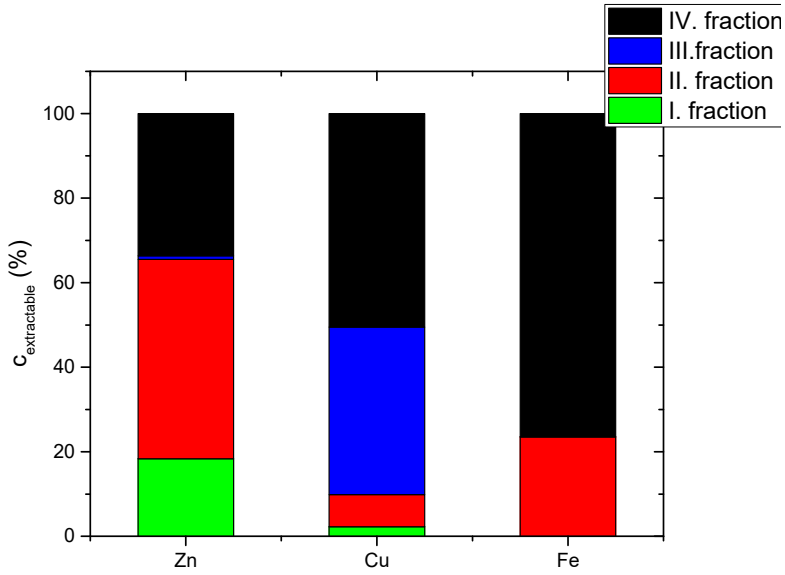


Figure 3. BCR sequential extraction protocols of Zn, Cu and Fe from PM. I. fraction represents water-extractable metal ions, exchangeable ions and ions bound in carbonates; II. fraction represents metal ions bound in Fe and Mn oxides; III. fraction represents metal ions incorporated in organic matter and sulfides; IV. fraction represents the residual fraction of metals.

To determine the ecotoxicity of water-extractable substances from the pyrolysis material, we conducted a test using small aquatic crustaceans, specifically the common daphnia (*Daphnia pulex*). The results obtained (Figure 4) did not confirm a statistically significant difference between the mortality of individuals after 48 h in 100% leachate of the PM compared to the control (with zero concentration of leachate). No statistically significant impact on mortality of small aquatic crustaceans was observed in our work; however, in their work, Zielińska and Oleszczuk [25] observed a negative impact of extractables from several samples of pyrolysis products on the mortality of Great Daphnia (*Daphnia magna*). Given that we did not observe a negative impact of the pyrolysis material on Daphnia pulex, we can conclude that the material is relatively safe, and the aqueous leaching of the material should not impact other aquatic organisms.

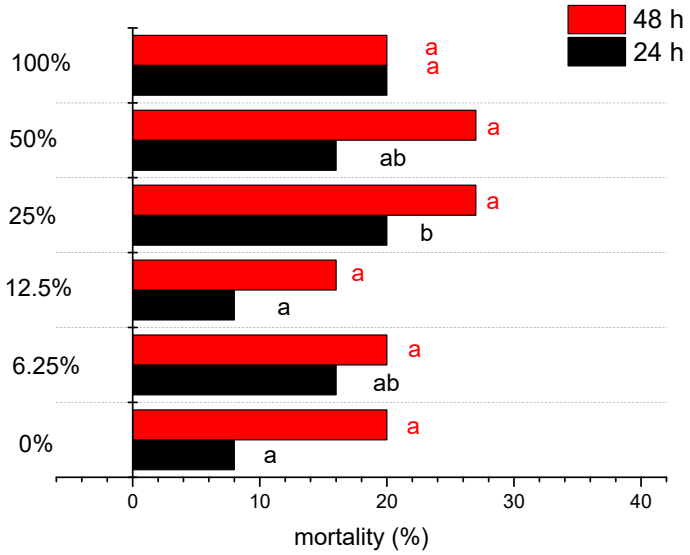


Figure 4. Comparison of the effect of water-extractable substances from PM on the mortality of *Daphnia pulex* with exposure periods of 24 h and 48 h. Percentages represent the proportion of PM aqueous extract in the culture medium. Different letters indicate statistical significance between additions to a given parameter based on the Tukey post-hoc test ($p < 0.001$).

To determine the bioavailability of plant objects, we selected lettuce (*Lactuca sativa*) as a model organism. Lettuce seeds were planted in substrates enriched with 5% PM addition (5% PM), 10% PM addition (10% PM), 20% PM addition (20% PM) and pure PM (100% PM). A common, commercially available horticultural substrate served as control (C). In the analysis of lettuce biomass, we focused on the presence of heavy metals Cd, Zn, Cu and Fe (Figure 5). The presence of Cd was not detected in any of the lettuce biomass (or was below the detection limit of the analytical method). The concentration values of Zn and Fe in lettuce biomass were close to < 2 mg/g. The highest Zn concentration was measured in lettuce biomass growing on substrate enriched with 5% PM. The observed Zn concentrations decreased as follows: 5% PM > 20% PM > 10% PM > C > 100% PM. For Cu concentrations, the order was as follows: 20% PM > 10% PM > 5% PM > C > 100% PM and for Fe concentrations: 20% PM > 5% PM > 10% PM > C > 100% PM. The values of the concentrations of the investigated heavy metals were the lowest at 100% PM, suggesting that a certain amount of the determined heavy metals was also present in the garden substrate (C). Likewise, metals present in PM due to substrate components could have been more mobilized by the change in pH and the presence of humic substances compared to 100% PM exposure. During the 60 days of monitoring the growth of sown lettuce, those samples that were planted in 100% PM performed significantly better. In their study, Tomczyk et al. [36] reported that PM added to soil positively affected the growth of sown cress (*Lepidium sativa*) compared to soil containing sewage sludge. They further reported that PM prepared at a higher pyrolysis temperature reduced the stimulatory effect of PM

on cress (*Lepidium sativa*). This is probably because the increased pyrolysis temperature reduces the proportion of amorphous organic matter in PM, thereby reducing its potential use as a fertilizer. Oh et al. [42] reported in their work that the addition of PM to soil improves the germination and growth of lettuce (*Lactuca sativa*) seedlings. In their study, Song et al. [43] investigated the possible effect of heavy metals contained in PM on the model organism garlic (*Allium sativum*) and found that garlic grew faster in soil amended with PM and had higher final dry matter yields compared to garlic from the reference soil, with the highest final yield recorded for PM, which was produced at 450 °C. Further, Song et al. [43] reported that garlic planted in soil amended with PM that was prepared at 450 °C contained the lowest heavy metal content compared to PM prepared at other temperatures (400 °C–550 °C). These results demonstrated that the accumulation of heavy metals from PM in plants can be inhibited by the correct choice of pyrolysis temperature.

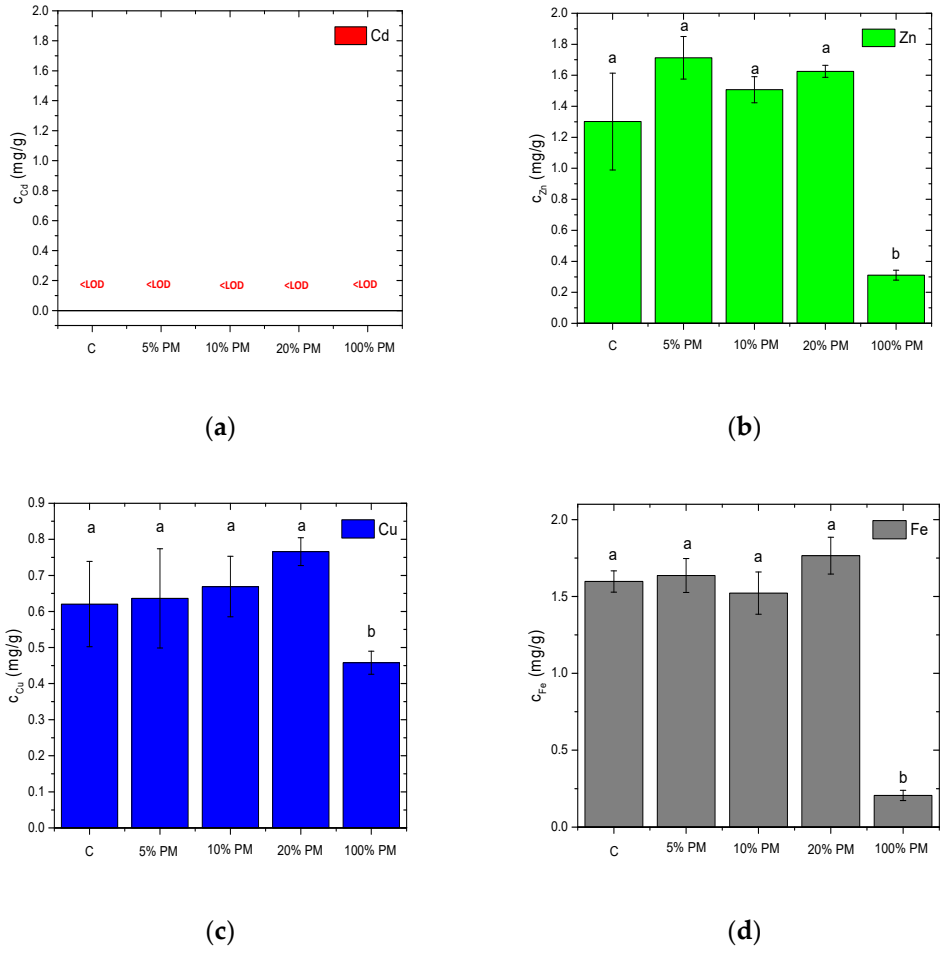


Figure 5. Concentrations of Cd (**a**), Zn (**b**), Cu (**c**) and Fe (**d**) quantified in dry biomass of Lactuca sativa lettuce after 60 days of cultivation at 60% water capacity, 12 h light/12 h dark, 22 ± 2 °C in unenriched garden substrate (C), substrate with 5% addition of PM (5% PM), substrate with 10% addition of PM (10%PM), substrate with 20% PM (10% PM) and in pure PM (100% PM). Different letters indicate statistical significance between additions to a given parameter based on the Tukey post-hoc test ($p < 0.001$).

4. Conclusions

The studied pyrolyzed material, prepared from municipal sewage sludge (PM), contained relatively high values of Fe concentration compared to other heavy metals studied. The other heavy metal concentrations in PM did not exceed the values that are established for land application of raw sewage sludge. The quantified concentrations of As, Hg and Cd in PM were below the detection limit of determination for the analytes (<2 mg/kg). Based

on the results of sequential extraction, we found that mobile forms of heavy metals (F1, F2) in the sewage sludge are transformed by the pyrolysis process into more stable forms of heavy metals (F3, F4) in PM. Bioavailability tests of the heavy metals Zn, Fe, Cu and Cd showed no toxicity hazard to lettuce (*Lactuca sativa* L.) and daphnia (*Daphnia pulex* L.); on the contrary, these selected model organisms thrived in PM-containing environments. From the results obtained, it can be concluded that pyrolysis is a suitable treatment process for sewage sludge that can effectively reduce the concentrations of mobile forms of heavy metals and thus reduce the bioavailability of heavy metals to soil ecosystems when used as an alternative fertilizer or soil additive.

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