ACS ENVIRONMENTAL Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Analysis of Organic Contaminants and In Vitro Cytotoxicity to Test the Suitability of External Organic Matter Processing

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Cite This: ACS Environ. Au 2025, 5, 376−386
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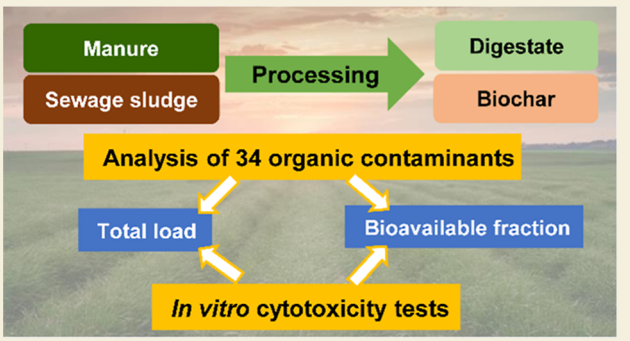
ABSTRACT: External organic matter (EOM), particularly from municipal waste, can contaminate soil when used to amend it. This may limit the benefits of using such an EOM to improve soil health and mitigate climate change. However, certain treatments may reduce the initial contaminant load of EOM. This study aimed to evaluate whether EOM processing can reduce its cytotoxicity and the concentration levels of 34 persistent and emerging organic contaminants. Sewage sludge and a mixture of manure and straw, processed by pyrolysis and anaerobic digestion to generate biochar and digestate, respectively, were selected for this study. An *in vitro* fish cell cytotoxicity test was performed to assess the toxicity of organic and aqueous extracts from the EOMs. It was found that organic contaminants are generally highly matrix-bound, resulting in low availability, reduced potential for leaching to groundwater, and effects on soil organisms after EOM application. The pyrolysis of sludge resulted in the almost complete removal of bisphenol A, tris(2-chloroisopropyl)phosphate, and octylphenol (removal ≥95%), while the concentration of the other contaminants monitored was reduced, with the exception of polycyclic aromatic hydrocarbons (PAHs) of lower molecular weight. In contrast, anaerobic digestion of manure did not result in a reduction of the contaminant load monitored except for bisphenol A. Cytotoxicity was also observed in aqueous extracts of manure but was reduced by anaerobic digestion. This suggests that anaerobic digestion could reduce potential hazards to groundwater or surface water from manure amendments. Organic EOM extracts were cytotoxic, indicating the presence of toxic products strongly adsorbed to these EOMs and retained in the soil after amendment.



KEYWORDS: *biochar, digestate, manure, pyrolysis, organic contaminants, cytotoxicity*

1. INTRODUCTION

According to Eurostat, the European Union (EU) generated 2.153 million tons of waste in 2020.[1] Reducing waste generation and, where unavoidable, promoting it as a resource and achieving higher recycling and safe disposal are long-term objectives of EU waste management policy. As part of the EU's Circular Economy Action Plan, the EU Fertilizer Regulation aims to bring novel fertilizer products onto the market, particularly those containing nutrients or organic matter from recycled biowaste. Using wastes rich in organic matter for the remediation of degraded soils is of great interest as it contributes to improving soil structure, controlling erosion, restoring depleted soil organic carbon, and reducing the emissions of the greenhouse gas, namely, carbon dioxide. This has important implications for both climate change mitigation and agricultural production.[2] However, despite great interest in using waste materials as organic amendments, external organic matter (EOM) may contain compounds that are undesirable for human or environmental health. For example, recent research has questioned the agricultural use of biosolids due to the presence of heavy metals and organic contaminants with risks of environmental transfer.[3] To enhance the quality and safety of their use, particular attention is being given to the treatment of waste. Consequently, certain treatments are anticipated to decrease the concentrations of contaminants in EOMs. The content and behavior of contaminants depend on the type of treatment (biological, chemical, thermal, or drying) applied to the EOM.

The presence of organic contaminants of emerging concern and their potential toxicity pose a challenge for recycling sewage sludge and manure as EOMs. While manure can be used either raw or treated, sewage sludge requires its treatment to reduce the levels of pathogens and produce a stable product

Received: September 12, 2024
Revised: January 31, 2025
Accepted: January 31, 2025
Published: April 10, 2025

before its application to soil. Common treatments for sewage sludge include anaerobic digestion, composting, thermal drying, and pyrolysis. The only limiting factor for the land application of sewage sludge in the EU is the concentration of heavy metals. However, a number of European countries have also imposed restrictions on organic pollutants, including nonylphenols, polycyclic aromatic hydrocarbons (PAHs), linear alkylbenzenesulfonates, and polychlorinated biphenyls (PCBs).[4] The recently implemented EU regulation on fertilizers sets maximum concentration limits for PAHs and impurities (glass, metals, and plastics) for certain fertilizer products.[5] It is essential to determine the levels of contaminants in EOMs and their effects on the environment.

Despite numerous studies investigating the use of waste as a soil amendment, significantly less attention has been paid to the potential occurrence of organic contaminants and their toxicological effects following waste treatment. Organic contaminants, including pesticides, PAHs, veterinary and human pharmaceuticals, bisphenol A (BPA), and other emerging contaminants, are likely to be found in sludge or manure. The multiresidue analysis of organic contaminants in sewage sludge and manure presents a significant challenge due to the diverse physicochemical properties of the compounds and the composition of the samples. Consequently, there has been a relatively low number of published studies on this topic.[6−8] Likewise, studies on the effect of different waste treatments on the content of organic contaminants have been scarce.

The integration of chemical and biological methods has been successfully applied to address different issues related to EOMs used as soil amendments. The evaluation of sewage sludge applied in agricultural lands was addressed by Bonomo et al.[9] using chemical evaluation and cytological effects in cell lines to determine its toxicity and relative contribution to environmental pollution when used as an agricultural soil amendment. The ecotoxicological evaluation, including the assessment of aqueous soil extracts, carried out in another study made it possible to conclude that organic amendments, such as compost or pig slurry, improved the properties of contaminated soils.[10] Similarly, based on the use of aquatic bioassays to test eluate extracts from nine different EOMs, Alvarenga et al.[11] found that toxic responses were more related to the degree of stabilization of the organic wastes, or the treatment used to achieve this stabilization, than to their chemical characterization. In this sense, solely determining the EOM contamination load, as required by existing regulations, may not be sufficient to adequately protect the environment. Integration with an ecotoxicological evaluation could provide a more truthful and holistic picture of the adverse effects that may result from their use on land. The above studies used different biological receptors (including plants, worms, and daphnids, among others) for ecotoxicological testing of both water and organic extracts of EOMs, but none used cell-based in vitro bioassays. These cell-based bioassays, also known as "bioanalytical tools", are an alternative system that offers a way forward for more comprehensive chemical assessment of complex mixtures[12] in a cost-effective manner and were used in our study as a complementary analysis for hazard characterization of EOMs.

In the present study, sewage sludge and manure were selected as the original EOMs and subjected to pyrolysis and anaerobic digestion to produce biochar and digestate. Based on our previous studies, 34 organic contaminants, covering both persistent and emerging contaminants with different physicochemical properties, were selected as target contaminants. In parallel, two cytotoxicity tests were performed using the RTG-2 cell line for assessing the cell viability and mitochondrial activity after exposure to both organic and aqueous EOM extracts. The primary hypothesis of this study is that the processing of the EOM would reduce the levels of the organic contaminants present in the raw EOM and the cytotoxicity of their extracts. The main objective of this work was to evaluate the efficacy of the two EOM treatments mentioned above in reducing the concentration of selected organic contaminants and cytotoxicity of EOM extracts.

2. MATERIALS AND METHODS

2.1. Reagents and Standards

Residue analysis-grade dichloromethane, acetonitrile, ethyl acetate, hexane, and acetone were purchased from Scharlab (Barcelona, Spain), and Bondesil-C18 silica (40 μm particle diameter) was acquired from the same source. A mixture of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA, purity ≥95%) with 1% *tert*-butyldimethylchlorosilane (TBDMCS), used as a derivatization reagent, and nonylphenol (4-*n*-NP, purity >97%) was obtained from Sigma-Aldrich (Steinheim, Germany). Standard solutions of a mixture of 18 PAHs (2000 μg/mL each), a mixture of deuterated PAHs (200 μg/mL), and a mixture of seven PCBs (10 μg/mL each) as well as standards of methylparaben (MeP), propylparaben (PrP), bisphenol A (BPA), bisphenol F (BPF), octylphenol (OP), tri-*n*-butyl phosphate (TBP, purity 99.5%), tris(2-chloroethyl)phosphate (TCEP, purity 98.5%), and tris(2-chloroisopropyl)phosphate (TCPP, purity 95.5%) were supplied by LGC Standards (Barcelona, Spain). The PAHs analyzed were 1-methylnaphthalene (1MeNaph), 2-methylnaphthalene (2MeNaph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), naphthalene (Naph), phenanthrene (Phen), anthracene (Anth), fluoranthene (F), pyrene (Py), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IcdPy), dibenzo[*ah*]anthracene (DBahA), and benzo[*ghi*]perylene (BghiP). The PCBs evaluated were PCB 28, 52, 101, 118, 138, 153, and 180.

The deionized water was obtained from a Milli-Q water purification system by Millipore (Bedford, MA, USA). Standardized aluminum oxide 90 supplied by Merck (Darmstadt, Germany) was subjected to activation at 180 °C for 24 h and deactivated using deionized water (3%, w/w) before use. Copper, provided by Panreac (Barcelona, Spain), underwent activation using 30% hydrochloric acid (3 M) for 1 min to eliminate the surface copper oxide. The copper was then washed successively with deionized water, acetone, and hexane.

Independent stock solutions of each compound were prepared at a 500 μg/mL concentration level in acetonitrile. A working standard mixture of all of the compounds at 5 μg/mL, except PAHs at 2.5 μg/mL, was prepared and stored in amber glass bottles. This mixture was used to prepare appropriate solutions as calibration standards. A standard solution containing all labeled compounds used as internal standards was prepared in ethyl acetate. All solutions were stored at −20 °C before use.

Minimal essential medium (1× MEM and 10× MEM), fetal bovine serum (FBS), L-glutamine, 1× MEM nonessential amino acids, 50 U/mL penicillin/50 μg/mL streptomycin, and trypsin−EDTA for cell culture were supplied by Biowest-Labclinics (Spain). Neutral Red (NR) dye was purchased from Sigma-Aldrich (Germany), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was supplied by Invitrogen (Spain).

2.2. EOMs and Treatments

2.2.1. Sewage Sludge and Biochar. Anaerobically digested and dewatered sewage sludge (80%) and waste wood (20%), collected from the Helsinki region (Finland), were treated with thermal drying and pyrolyzed at 565 °C for 75 min in a pilot pyrolysis plant operated

https://doi.org/10.1021/acsenvironau.4c00092
ACS Environ. Au 2025, 5, 376−386

by Helsinki Region Environmental Services (HSY) to produce the biochar sample (https://www.hsy.fi/en/sludge-char-project/process).

2.2.2. Manure and Digestates. The cattle manure and wheat straw feedstocks used in this study were sourced from Luke's research farm in Jokioinen (Finland). They were anaerobically digested under mesophilic conditions at a mass ratio of 84%/16% mass ratio (manure/straw) as described in Tampio et al.[13] The digestion took place in two parallel 1 m^3 batch-type leach-bed reactors (reactors R1 and R2), where the leachate liquid was circulated through the solid substrate batch, resulting in the production of digestate R1 and digestate R2 samples. The reactors were filled with the feedstocks in multiple layers, starting with straw. The percolation liquid flow was gradually intensified during the reactor runs to circulate 10 L of liquid every 48, 24, 12, 6, 4, and 2 h. After 65 days of operation, reactor R2 was switched to manual feeding every 24 h with breaks over the weekends. Tap water was added to the percolation liquid tanks to maintain a constant liquid volume within the percolation tanks. In total, 207 and 229 L were added to reactors R1 and R2, respectively. The reactor experiment lasted for 139 d. At the end of the experiment, the reactors were drained of liquid within the biomass and reactor bottom. The cumulative gas production was 138 and 115 L CH_4/kg VS in reactors R1 and R2, respectively.

2.3. Sample Preparation for Organic Contaminant Analysis

2.3.1. Determination of Total Load of Organic Contaminants (Organic Extract). The analytical procedure employed was based on a previously published paper with slight modifications.[8] Briefly, 3 g of deactivated alumina and 2 g of activated copper powder were mixed in a glass mortar with 2 g of the sample (sewage sludge, biochar, manure, digestate, or straw) spiked with 50 μL of the mixture of internal standards. The blended mixture was then transferred into a 20 mL glass column, which was equipped with a cellulose frit at its end. Then, 6 mL of dichloromethane were added as an extraction solvent. The columns were then placed in an ultrasonic water bath at room temperature for 10 min, and the resulting extract was collected in a graduated glass tube. The extraction step was repeated with another 6 mL of dichloromethane, and the collected extracts were concentrated to 0.5 mL using a Genevac EZ-2 centrifugal evaporator (Net Interlab, Spain). They were then diluted to 2 mL with acetonitrile. Purification of the extracts was conducted using a 5 mL glass column containing C18 (2 g). The target compounds were eluted using 10 mL of acetonitrile, which was subsequently concentrated to 1 mL. Gas chromatography−tandem mass spectrometry (GC−MS/MS) was used to analyze the resulting organic extract.

2.3.2. Determination of (Bio)available Fraction of Organic Contaminants (Aqueous Extract). EOM aqueous extracts were obtained by placing samples in centrifuge tubes together with Milli-Q water at a ratio of 1:10 (w/v). This ratio was used for digestate and manure samples, but for biochar and sludge samples, the sample/water ratio was 1:6 (w/v). Samples were allowed to rotate on an overhead shaker for 24 h at room temperature. The tubes were then centrifuged at 5000 rpm for 15 min, and the supernatants were collected and filtered through 0.45 μm pore nylon membrane filters.

Liquid−liquid extraction of the aqueous extract was required before its GC−MS/MS analysis. An aliquot of the aqueous extract (4 mL) was spiked with 20 μL of the internal standard mixture. Then, 2 mL of dichloromethane were added, vortexed for 1 min, sonicated for 5 min, and centrifuged for 5 min at 5000 rpm and 20 °C. The organic phase (bottom layer) was collected with a syringe. The extraction was repeated with 2 mL of dichloromethane. The combined extracts were evaporated to dryness and reconstituted into 1 mL with acetonitrile before the chromatographic analysis.

2.4. Sample Preparation for Ecotoxicological Evaluation

The organic and aqueous extracts were obtained by following the procedure outlined in the Sample Preparation for Organic Contaminant Analysis section. However, the organic extract was obtained without adding the internal standard to the sample, and the final extract was reconstituted in 200 μL of methanol. For the aqueous extracts, an additional filtration step of 0.22 μm was required to ensure sterile conditions for cytotoxicity testing.

2.5. GC−MS/MS Analysis

Analyses were performed on an Agilent 7890A gas chromatograph coupled to a tandem triple quadrupole mass spectrometer, model 7000. A ZB-5MS fused silica capillary column, with 5% phenyl-polysiloxane as a nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25 μm film thickness) from Phenomenex (Torrance, CA, USA), was used. Helium at a constant flow rate of 1.2 mL/min (purity 99.995%) was employed as the carrier gas. Two-layer sandwich injections of 1 μL of sample and 0.5 μL of MTBSTFA with 1% TBDMCS were performed in pulsed splitless mode at 25 psi until 0.5 min with the splitless injector purge valve activated 1 min after sample injection (purge flow to split vent 60 mL/min). The column temperature was initially set at 80 °C (held for 0.5 min), increased at 8 °C/min to 230 °C, and finally programmed at 5 °C/min to 280 °C (held for 7 min). The run was performed with a solvent delay of 5 min. The total analysis time was 36.25 min.

Analyses were performed in the multiple reaction monitoring (MRM) mode with two transitions (precursor/product ion pair) for each analyte; one was used for quantification, and the other was used for identity confirmation. The precursor and product ions selected for each MRM transition, with their optimal collision energies, are summarized in Table S1. Analytes were confirmed by retention time and target and qualifier transition identification. Retention times must be within ±0.2 min of the expected time, and the ratio of qualifier to target must be within a range of 20% for a positive confirmation.

2.6. Cytotoxicity Testing

The RTG-2 fish cell line (ATCC, CL No. 55) was used to evaluate the cytotoxicity of aqueous and organic extracts obtained from waste (sewage sludge and manure) and their products after pyrolysis and anaerobic digestion (biochar and digestate, respectively). All culture method specifications are described in Sánchez-Argüello et al.[14]

Cells were seeded in 96-well microplates and allowed to attach under the same culture conditions (20 °C in 5% CO_2 in air). The cell monolayer was allowed to grow exponentially for 24 h and then treated with the organic extract (1%, 0.5%, and 0.25%) in 1× MEM or with the aqueous extract (75%, 50%, and 25%) in 10× MEM (25%). The total well volume (200 μL) for the 50% and 25% aqueous treatments was completed with sterile Milli-Q water (50 and 100 μL, respectively). Each treatment with the organic and aqueous extracts was replicated eight times per plate (one column). In addition, two columns of 1× MEM were used as a control (growth medium), one column of 1% methanol as a solvent control in the case of organic extracts, or one column of 10× MEM (25%) with sterile Milli-Q water (75%) as a blank in the case of aqueous extracts. Two experiments were performed using two different cytotoxicity tests based on Sánchez-Argüello et al.,[14] briefly described as follows: mitochondrial activity was evaluated by tetrazolium salt reduction (MTT assay), while cell viability was assessed as neutral red uptake (NRU assay). The MTT assay is based on the measurement of mitochondrial activity by detection of the soluble yellow MTT tetrazolium salt to a blue insoluble MTT formazan product by the mitochondrial succinate-dependent dehydrogenase. The NRU assay is based on the uptake of the neutral red dye into the lysosomes of viable cells. MTT and NRU assays were based on Sánchez-Argüello et al.[14] and consisted of the addition of MTT or neutral red dye solution to cells after treatment at 500 or 40 μg/mL and incubation for 4 or 3 h, respectively. Absorbance was measured at 570 nm for the MTT assay and 550 nm for the NRU assay.

2.7. Statistical Analysis

Statistical analysis of the data was performed using STATGRAPHICS software (version XVII). Significant differences between treatments were determined by one-way analysis of variance (ANOVA) using Fisher's least significant difference (LSD, $P < 0.05$).

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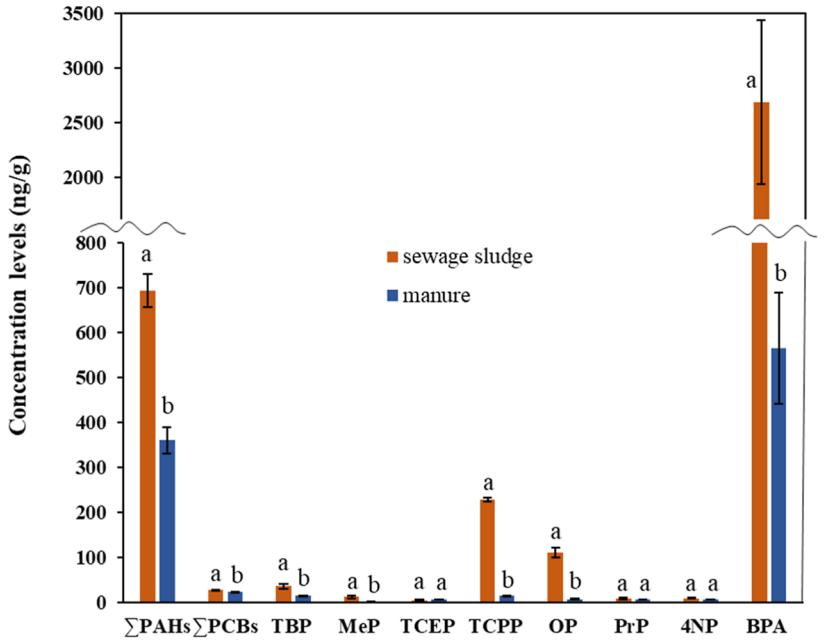

Figure 1. Mean concentration (ng/g, n = 3) of organic contaminants found in EOMs before processing (sewage sludge and manure). Different letters indicate significant differences (LSD test, $P < 0.05$).

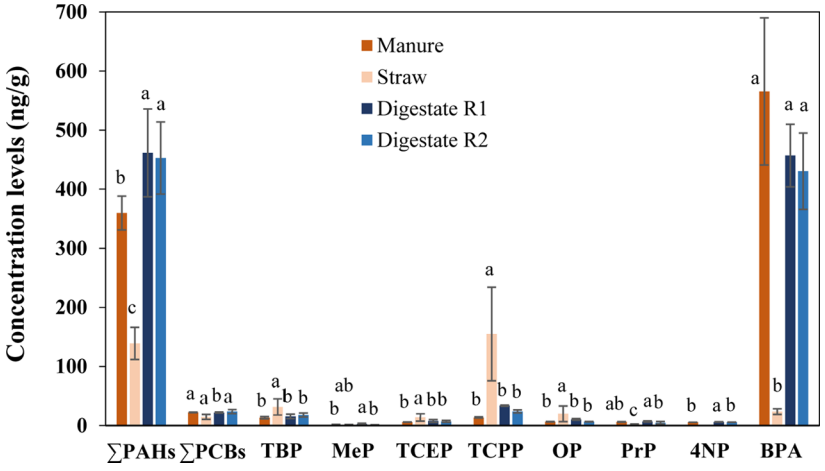

Figure 2. Mean concentration (ng/g, n = 3) of the organic contaminants found in manure, straw, and digestates. Different letters indicate significant differences (LSD test, $P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Organic Contaminant Content in Original EOM: Sewage Sludge and Manure

All organic contaminants tested, except BPF, were present in both original EOMs analyzed. Sewage sludge is produced by treating wastewater from many sources, so a wide range of organic contaminants may be present.[15] Manure, in contrast, is derived from livestock. Based on the origin of the untreated samples, lower concentrations of the target organic compounds were expected in manure than in sludge. This hypothesis was confirmed by the results obtained, as shown in Figure 1. BPA and PrP were two of the organic contaminants previously detected in poultry manure samples.[16] The results obtained are summarized in Tables S2 and S3, which showed that BPA was the analyte found at higher concentration levels in both EOMs, although the BPA content in sludge was significantly higher (2688 ± 745 ng/g) than in manure (565 ± 124 ng/g). Significant differences were also observed in the concentration levels of TCPP, OP, TBP, MeP, and the sum of PAHs (see Figure 1).

3.2. Effect of Treatment Processes on the Organic Contaminant Content

The concentrations of the organic contaminants found in manure, straw, and the digestates obtained are given in Table S2 and depicted in Figure 2. The results obtained show that the contaminant load in the digestates is closely related to the manure content (which represents 84% of the sample before processing) and that few differences were observed in the organic contaminant content between the two digestate samples. Regarding the straw, the presence of PAHs and TCPP was noted. PAHs are ubiquitous pollutants that can reach the plant from the air or through its root system. The accumulation of PAHs in cereal plants in PAH-contaminated soil was recently confirmed by Cai et al.[17] In a field study carried out by Li and Ma,[18] the uptake of PAHs by wheat

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ACS Environ. Au 2025, 5, 376−386

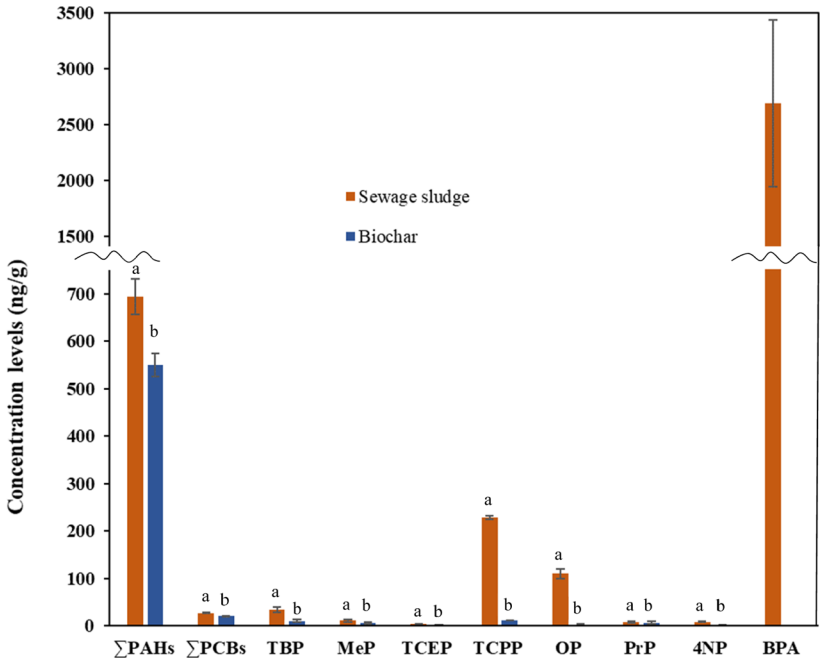

Figure 3. Mean concentration levels (ng/g, $n = 3$) of organic contaminants found in sewage sludge and biochar. Different letters indicate significant differences (LSD test, $P < 0.05$).

sewage sludge-amended soils was evaluated, with PAH levels in straw ranging from 110 to 260 ng/g dry weight. These levels are similar to those found in the straw analyzed in the present study. The organophosphate TCPP was found at higher levels in straw than in manure. In a study that assessed the uptake of organophosphates and other emerging pollutants, a high uptake of TCEP and TCPP was observed in plants, with the highest concentrations in straw.[19] More recently, TCPP and TCEP were reported to be the two dominant organophosphate ester congeners detected in field plant samples.[20] Some compounds present only in the manure, such as Phen, 4-NP, or DBahA, were also quantified in the digestates at similar concentration levels. On the contrary, the content of low molecular weight PAHs (Naph, 1MeNaph, Anth, and Phen) increased significantly after treatment (see Table S2). Consequently, the sum of PAH content in the digestates was higher than in manure but not significantly different (461, 453, and 365 ng/g for digestate R1, digestate R2, and manure, respectively). Information about the effect of anaerobic digestion on organic contaminants is scarce in the available literature. Therefore, for many of the compounds included in our study, there is no published information on the effect of anaerobic digestion on their concentration levels that would allow us to compare the results obtained in different studies. Nevertheless, our results agree with those reported by Brändli et al.,[6] who investigated the fate of PCBs and PAHs during composting and digestion in a full-scale plant. They concluded that PCB and PAH concentrations were not affected by the digestion process. Our findings of similar concentrations of BPA in both original and treated EOM agree with other studies[21] that reported that no biodegradation of BPA was observed during anaerobic digestion of municipal solid waste. According to their research, the decrease in BPA concentration was the result of its adsorption to solid waste rather than biodegradation. This observation may provide an explanation for why BPA is frequently detected at high levels in landfill leachates and could exist at high concentrations in anaerobic digestates. The presence of MeP and PrP in anaerobic digestate based on food waste was first described by Golovko et al.[22] with average concentrations of 11 and 23 ng/g dw, respectively, with MeP being the paraben found in the highest concentration in sewage sludge in Sweden and in China. Both parabens were found in manure, straw, and digestate (see Table S2 and Figure 2) at average concentrations slightly lower than those reported by Golovko et al.[22] No reduction in the concentrations of these compounds was observed after anaerobic digestion.

Pyrolysis of sewage sludge to produce biochar was evaluated for its ability to reduce the concentration of organic contaminants. As shown in Figure 3 and Table S3, pyrolysis resulted in a significant decrease in the concentrations of all the evaluated organic contaminants present in sewage sludge, except for Naph and its derivatives, 1MeNaph and 2MeNaph, which increased by 51%, 37%, and 63%, respectively. The biochar was produced from a mixture of sewage sludge and waste wood, which was not analyzed. Löser et al.[23] reported that if waste wood is protected using tar oil, this could contribute to the concentration of certain PAHs in the waste. However, the waste wood fraction used in this study was not treated with tar oil. As wood waste accounted for only 20% of the mixture used for biochar production, sewage sludge was expected to be the primary source of organic pollutants. During biochar production, the formation of PAHs is inevitable,[24] as well as other potentially toxic organic compounds.[25] This could explain the observed increase in Naph, 1MeNaph, and 2MeNaph. The results obtained are consistent with those reported by Zielińska and Oleszczuk,[26] who observed that the concentration of all the individual PAHs decreased with respect to that of the initial sewage sludge, except for Naph, whose concentration increased from 54.2% to 92.6% in biochar produced at 500/600 °C and by about 34% in biochar produced at 700 °C. In addition, these authors evaluated the effect of sewage sludge pyrolysis on the content of PAHs and concluded that free PAHs were present in

https://doi.org/10.1021/acsenvironau.4c00092
ACS Environ. Au 2025, 5, 376−386

Table 1. Summary of Organic Contaminant Concentration Levels (ng/g, n = 4) Found in the Aqueous Extracts[a]

	manure		digestate R1		digestate R2		sewage sludge		biochar	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Naph	61.3[a]	18.2	55.3[a]	13.3	59.0[a]	7.9	62.3[a]	0.2	47.8[a]	9.6
1MeNaph	15.2[a]	0.5	14.1[a]	0.9	17.2[a]	4.1	9.9[a]	1.3	14.2[a]	4.0
2MeNaph	4.0[a]	0.4	3.8[a]	0.8	5.6[a]	2.6	5.9[a]	0.4	7.2[a]	2.2
Ace							1.1[a]	0.1	1.1[a]	0.2
Fl							1.0[a]	0.1	2.5[a]	1.1
TBP							1.7[b]	0.1	3.8[a]	1.2
MeP	0.7[a]	0.1	1.0[a]	0.3	0.7[a]	0.2				
Anth	2.8[a]	0.4	2.9[a]	0.4			0.6[a]	0.1	0.3[a]	0.2
Phen							4.6[a]	0.6	5.2[a]	1.7
TCPP	13.0[b]	1.3	36.9[a]	3.6	36.5[a]	3.7	1.4[a]	0.2	3.0[a]	2.0
OP	4.3[b]	0.3	6.2[a]	0.6	4.7[b]	0.2	1.5	0.2		
PrP	2.6[a]	0.1	2.8[a]	0.1	1.8[a]	1.4				
Py	0.4[b]	0.2	0.5[a,b]	0.03	0.6[a]	0.1	1.3[a]	0.2	0.7[b]	0.3
F	1.2[b]	0.3	1.3[a,b]	0.1	1.6[a]	0.3	1.6[a]	0.2	1.0[a]	0.3
BPA	22.0[b]	8.3	8.3[c]	3.3	150.6[a]	5.5	3.9	0.5		
BbF +Bkf							0.4	0.1		

[a]Different letters indicate significant differences between treatments (LSD test, $P < 0.05$).

biochar at concentrations several times lower than in sewage sludge and that 5- and 6-ring PAHs were not detected in biochar.[27] Buss et al.[25] found that Naph was the compound with the highest content in different biochar samples of all individual PAHs (see Table S3). In contrast, Alipour et al.[28] reported the complete removal (≥99.9%) of PCBs, PAHs, and PPCPs by pyrolysis at 600 °C of two different sewage sludge samples for biochar production. In our study, although pyrolysis was performed at a similar temperature (565 °C), only BPA and DBahA were completely removed, but the concentrations of total PAHs, total PCBs, and other organic compounds, such as the organophosphate esters (mainly TCPP), were significantly reduced in concentration after pyrolysis treatment. The concentrations found in the sludge (before treatment) and in the biochar (after treatment) are shown graphically in Figure 3. In this context, it is important to highlight the significant reduction of the BPA content from 2688 ng/g to no detection. This result is noteworthy because BPA was present in sewage sludge at very high concentrations,[29,30] posing a significant risk to soil health and discouraging its direct use as an agricultural amendment. Kumagai et al.[31] reported that after 30 min at 320 °C, BPA decomposes almost completely. Volatilization or degradation may be responsible for the reduction of the organic contaminants after pyrolysis.[32]

3.3. Evaluation of the (Bio)availability of Target Compounds

The organic extract of EOM gives an estimate of the maximum amount of organic contaminants. Sorption is considered a key process in the mobility, bioavailability, and degradation of contaminants in the environment. The physicochemical properties of the EOM and the characteristics of the compounds have a significant effect on this process. Bioavailability can be defined as the fraction of a chemical compound in the EOM that can be absorbed or transformed by plants or other living organisms.[33] To assess the bioavailability of the organic contaminants studied, it is necessary to determine their concentrations in the aqueous extract of the EOM. Therefore, it is important to compare the concentrations of the target compounds in the aqueous extracts of original and processed EOMs. Aqueous extracts, obtained as indicated in the sample preparation, were analyzed to determine the concentration levels of the target analytes. Table 1 presents the results of the detected contaminants in the aqueous extracts.

Naph and its derivatives, 1MeNaph and 2MeNaph, were detected at high concentrations in the aqueous extracts before and after the pyrolysis of the sludge. However, their availability was slightly lower in the biochar sample. The more volatile PAHs were the most available, while the heavier PAHs showed very low or no availability. PCBs, TCEP, and 4NP were not detected in any of the aqueous extracts. Data on the concentration of organic contaminants in aqueous extracts from soil, sludge, or manure are scarce in the available literature. Previous studies[34,35] have shown that the availability of PAHs and PCBs in soil is low and highly dependent on soil physicochemical properties and organic matter content. In the case of the organophosphates, TCPP was detected at very low concentrations in all samples, while TCEP was not detected in any of the EOM aqueous extracts. Considering the total concentration of TCPP presented in Tables S2 and S3, it is evident that it is highly available in manure and digestates (approximately 100% of the total content). In contrast, TCPP availability is low in sewage sludge and biochar, particularly in sewage sludge, where only 0.6% of the total content is available. TBP, the other organophosphate evaluated, was found at low levels only in the aqueous extracts of sewage sludge and biochar, comprising 4.8% and 37% of their total content, respectively. These results are consistent with those reported by Cristale et al.,[36] who studied the sorption/desorption behavior of seven organophosphate esters, including TCPP, TCEP, and TBP, on soils with different characteristics. The authors suggest that the matrix may play a significant, albeit unclear, role in the availability of organic contaminants. They report that the sorption of the most soluble compounds, namely, TCEP and TCPP, was found to be dependent on the content of soil organic matter, with desorption rates exceeding 50%. In contrast, less water-soluble compounds, such as TBP, were completely sorbed and did not undergo desorption.

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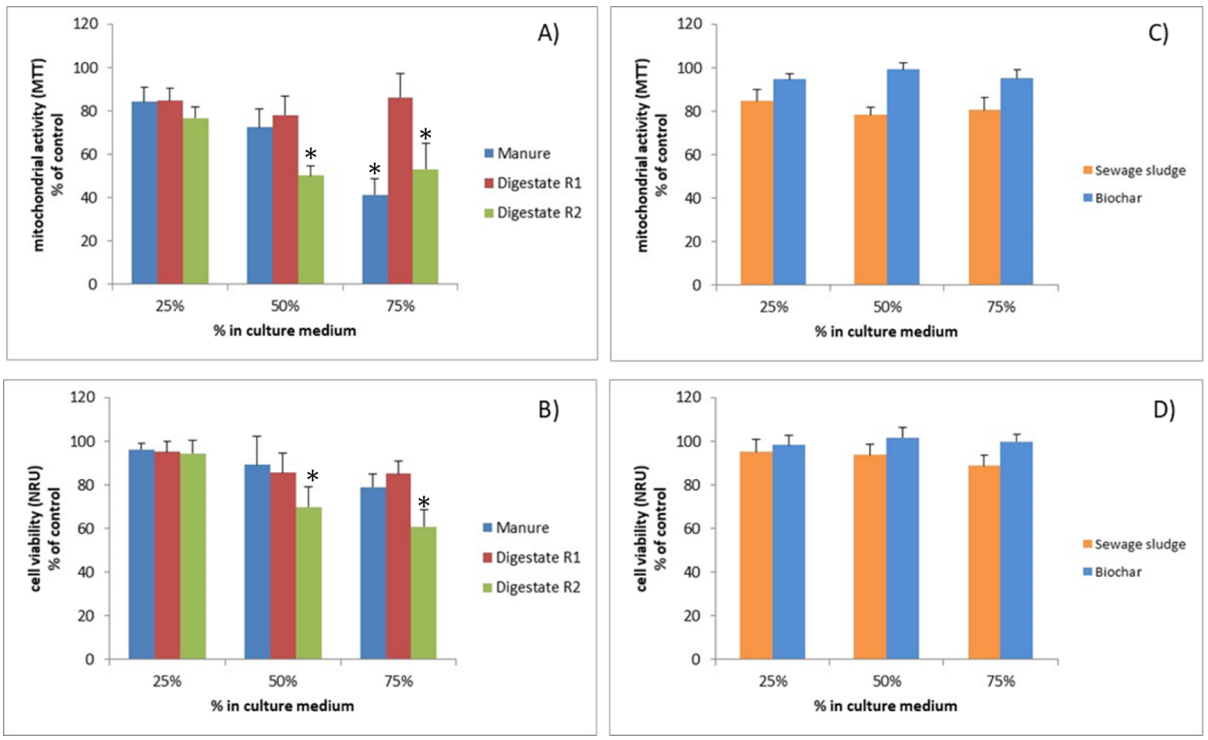

Figure 4. Cytotoxicity of aqueous extracts: manure and digestate (R1 and R2) for the MTT assay (A) or NRU assay (B) and sewage sludge and biochar for the MTT assay (C) or NRU assay (D). Cellular responses are expressed as a percentage of the growth medium. The percentages of extracts in cell culture medium (10× MEM) were 25%, 50%, and 75%. *Significant differences compared to control [10× MEM (25%) and sterile Milli-Q water (75%)] $p < 0.05$ ANOVA analysis.

The availability of OP was influenced by the matrix, as shown in Table 1. Despite the high concentration (100 ng/g) detected in sewage sludge, only 1.4% of OP was available, compared to 66%, 60%, and 72% of OP leached from manure, digestate R1, and digestate R2, respectively. The matrix had a significant impact on the availability of BPA. Despite the high concentration found in sewage sludge (2688 ng/g), the percentage of BPA in the aqueous extract of sewage sludge was only 0.1%. However, the leachability of BPA was similar after anaerobic digestion of manure, resulting in comparable BPA concentrations in both manure and digestate R1 aqueous extracts. In contrast, the concentration of BPA in the aqueous extracts of digestate R2 was about 20 times higher than in digestate R1. It is important to highlight that after 65 d of operation, the percolation liquid circulation in reactor 2 was switched to a less frequent cycle (from a 2 to a 24 h feeding cycle). It is possible that the BPA, a relatively water-soluble compound, started to accumulate in the digestate R2 due to the less frequent circulation of the percolate liquid in reactor R2 compared to that in reactor R1. However, it was not possible to analyze the percolate liquid.

3.4. Cytotoxicity of Original and Processed External Organic Matters

Despite the importance of chemical parameters for the assessment of potential environmental or human risks of EOM application on agricultural land, some authors have pointed out that the contaminant load of a material is sometimes not related to its toxicity.[11,37] For this reason, the integration of chemical and biological analyses is a powerful strategy to deal with the complexity of EOM samples. Ecotoxicological testing can detect the effects of the bioavailable fraction of any cocktail of compounds in EOMs, including their additive, antagonistic, and synergistic interactions. With this in mind, we selected a high-throughput *in vitro* assay using fish-derived cells suitable to assess the cytotoxicity of aqueous and organic extracts of EOMs. The cytotoxicity assessment of aqueous extracts on aquatic organisms can be extrapolated to water quality affected by leaching or runoff of contaminants contained in applied EOMs and indirectly to the contaminant retention of EOMs. However, the more lipophilic contaminants (i.e., organic contaminants) that are not transferred to the water compartment will be sorbed to the soil and may then be bioavailable or bioaccumulated by soil organisms. The cytotoxicity assessment of organic extracts can then be linked to the presence of organic contaminants in soils from EOMs, as demonstrated here and proposed by other authors.[9]

3.4.1. Original EOMs: Manure and Sewage Sludge.

Cytotoxicity for aqueous manure extracts showed that the only statistically significant cellular effect observed was MTT reduction at the highest percentage of extract in the culture medium (75%). This effect disappeared with the next dilution of the extract (50% in the culture medium; Figure 4A). Nevertheless, the NRU assay was less sensitive than the MTT assay as a slight reduction in cell viability was observed for the highest percentage of aqueous manure extract (Figure 4B). In contrast, none of the dilutions of sewage sludge aqueous extracts produced cytotoxicity in either the MTT or NRU assays (Figure 4C,D). These results showed that the cytotoxicity of bioavailable compounds present in aqueous extracts was higher for manure than for sewage sludge. Therefore, the use of this type of manure as an amendment without any treatment could have an impact on the surrounding water bodies due to the presence of water-

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ACS Environ. Au 2025, 5, 376−386

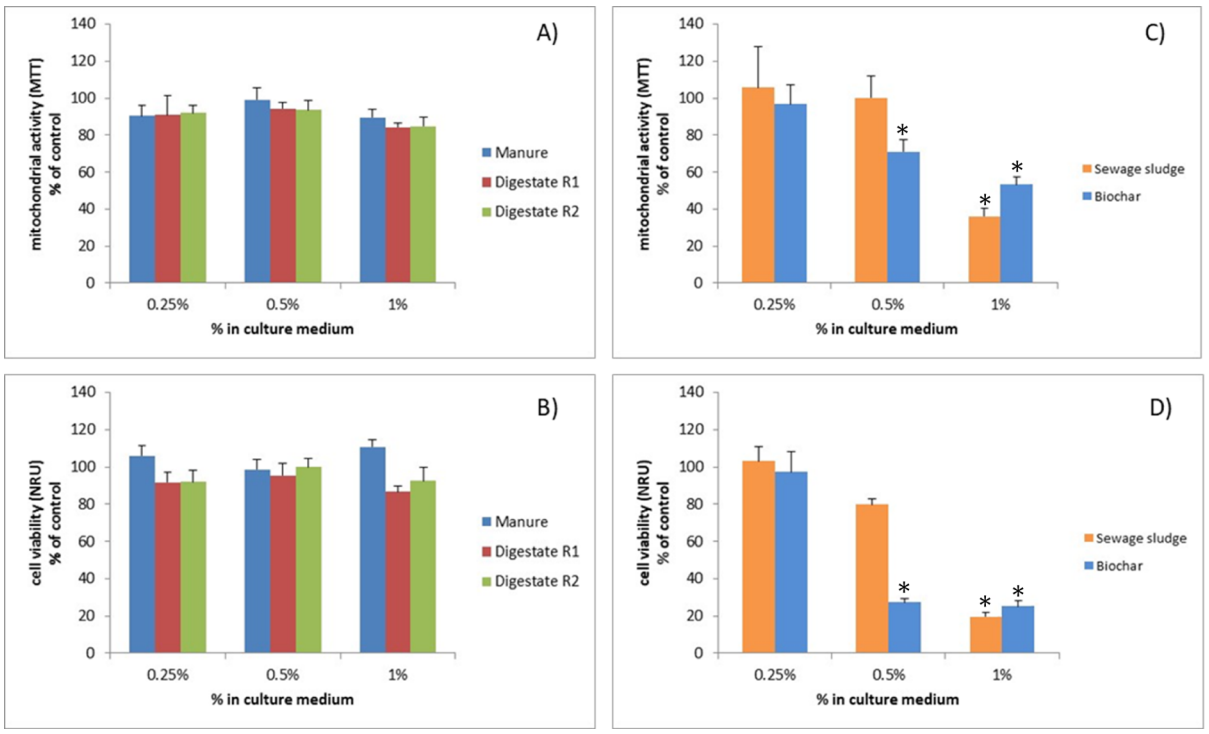

Figure 5. Cytotoxicity of organic extracts: manure and digestate (R1 and R2) for the MTT assay (A) or NRU assay (B) and sewage sludge and biochar for the MTT assay (C) or NRU assay (D). Cellular responses are expressed as a percentage of the growth medium. The percentages of extracts in cell culture medium (1× MEM) were 0.25%, 0.5%, and 1%. *Significant differences compared to control (1% methanol) $p < 0.05$ ANOVA analysis.

extractable toxic compounds. Nevertheless, there was no cytotoxicity for the organic extracts from manure (Figure 5A,B), suggesting no sorption of toxic compounds to its organic fraction. On the contrary, statistically significant cytotoxic effects were observed for the organic sewage sludge extracts, which were maximum at the highest percentage of the extract in the culture medium (1%) and disappeared with the next dilution (0.5%) in the case of sewage sludge but not in the case of biochar (Figure 5D). This may indicate a greater retention of organic contaminants by sewage sludge than by manure, as would be expected for this material derived from wastewater treatment. It can be concluded that both untreated EOMs could have ecotoxicological effects but of different natures: sewage sludge related to toxic compounds immobilized in soil and manure related to potentially leached toxic compounds. Although sewage sludge often contains higher concentrations of potentially toxic compounds than manure,[38] information on whether these toxic compounds are readily solubilized or, on the contrary, strongly adsorbed to EOMs can provide important information on their bioavailability, persistence, or potential bioaccumulation. Our experimental approach of testing the cytotoxicity of both aqueous and organic EOM extracts can be a useful tool for this purpose.

3.4.2. Effect of Treatment Processes on the Cytotoxicity. The cytotoxicities of aqueous extracts of the two products obtained after digestion of manure (digestates R1 and R2) were different. While digestate R1 had no effect on MTT or NRU assays (Figure 4A,B), digestate R2 reduced the mitochondrial activity by almost 50% at the lowest dilution in culture medium in a statistically significant manner (Figure 4A). The biogas production rate and degradation had differences between the two reactors (R1 and R2), caused by changes in the percolation liquid circulation. This may have resulted in an incomplete degradation of digestate R2 compared to that of digestate R1, which can have influenced the presence of toxic compounds. The observed cytotoxicity of digestate R2 was maintained at the next dilution (50%), indicating that digestate R2 was more toxic than manure. On the contrary, manure treatment in reactor R1 produced a digestate R1 with reduced cytotoxicity compared to manure, demonstrating that anaerobic digestion was efficient in reducing the toxic load of original EOM. According to the results of the organic compounds measured here, the only difference observed between reactors R1 and R2 corresponded to the BPA component (Table 1), which could be responsible for the differences in cytotoxicity between digestates R1 and R2, but it could not be excluded that other organic compounds not measured or even other chemicals such as metals could be the origin of digestate R2 cytotoxicity. Finally, as expected, there was no toxicity in the organic extracts from the digestates as none was observed in the corresponding manure (Figure 5A,B).

A comparison of the cytotoxicities of sewage sludge and its corresponding biochar showed slight differences between the two EOMs. Aqueous extracts were not cytotoxic in any case (Figure 4C,D). However, the organic extracts of both EOMs were cytotoxic at the highest concentration used (1%), and higher dilutions (0.5% and 0.25%) were required to reduce the cytotoxicity of the organic extracts of biochar (Figure 5D). These effects suggest that biochar had more toxic compounds in its organic fraction than in sewage sludge. In fact, it has been described that biochar production can generate potentially toxic organic pollutants including polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.[39]

To summarize, anaerobic digestion was able to reduce the presence of toxic compounds from aqueous extracts in manure

https://doi.org/10.1021/acsenvironau.4c00092
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when the reactor operated under optimal conditions (reactor R1). The same was observed in other studies as anaerobic digestion using manure as a feedstock potentially reduced ecotoxicity.[40] On the other hand, the ecotoxicity of the sewage sludge extracts was only observed in the organic extracts and was not significantly reduced by pyrolysis. Although pyrolysis can reduce the toxicity of aqueous sludge extracts[41] and increase the stability of potentially toxic elements such as metals, it can also increase the mobility and bioavailability of organic and inorganic contaminants in sewage sludge.[42] In our study, the differences in contaminant mobility and bioavailability were not caused by the treatment process but rather by inherent characteristics of the sludge sewage as the organic fraction of pre- and postprocessed sludge sewage was always toxic, again highlighting the importance of testing the cytotoxicity of not only the aqueous extractable contaminants but also of the organic extractable contaminants.

The use of cytotoxicity testing of EOM extracts represents a rapid and simple ecotoxicological assessment that can complement chemical analysis to provide a measure of the EOM's potential environmental impact. Nevertheless, it must be emphasized that the intrinsic toxicity of EOMs observed in extracts obtained in the laboratory is likely to be different when EOMs are applied under field conditions, where many other factors have an influence (e.g., soil properties, degradation, leaching, and mineralization). The results demonstrated the feasibility of testing the effect of EOM treatments on reducing the presence of toxic compounds in such complex mixtures. General statements about the efficiency of EOM treatments cannot be made from this study as only two different feedstocks and treatments used to produce EOMs used as soil amendments were tested. Despite these limitations, as bioassays are essential to capture the effects of all chemicals in mixtures,[12] our testing strategy could be used in the initial screening of different EOM treatment technologies. Although most ecotoxicological studies of the effects of EOMs use aqueous extracts to assess the effects of contaminant leaching, this methodology ignores the presence of organic contaminants that are strongly adsorbed on organic matter. Both aqueous and organic extracts represent the total contamination load in EOMs, and a better predictive evaluation could be performed considering the ecotoxicological assessment of both extracts, as used in effect-based monitoring programs for wastewater samples.

4. CONCLUSIONS

The effect of pyrolysis and anaerobic digestion treatments on the concentrations of 34 organic contaminants and the cytotoxicity of organic and aqueous extracts was investigated in selected EOMs. The results suggest that the target contaminants were found in the EOM at concentrations of nanograms per gram and are strongly bound to the matrix, as evidenced by the low concentration levels detected in their aqueous extracts. Therefore, their limited availability reduces the risk of soil contamination following EOM application. The pyrolysis of sludge resulted in the almost complete removal of BPA, TCPP, and OP (removal ≥95%), while the concentration of the other contaminants monitored was reduced by 10−78%. However, there was an increase in the levels of Naph and its metabolites following pyrolysis. In the case of manure, there was no significant reduction observed following anaerobic digestion. Nevertheless, the digestion process reduced the cytotoxicity observed (i.e., mitochondrial activity) in manure, whereas pyrolysis did not mitigate the observed cytotoxicity in sewage sludge. The effectiveness of EOM processing gave different results depending on the analytical approach (chemical or biological), highlighting the need for a multidisciplinary strategy to investigate the environmental risk of EOMs for soil amendment. Our experimental approach of testing the cytotoxicity of aqueous and organic EOM extracts can be a useful tool to provide important information on the behavior of contaminants, thereby improving the information on the effect of the waste treatment on their availability and toxicity. Further research is required to enable the development of optimal management strategies for the processing and utilization of different EOMs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsenvironau.4c00092.

GC−MS/MS parameters; concentration levels of target contaminants in manure, straw, and resulting digestates; and concentration levels of target contaminants in sewage sludge and resulting biochar (PDF)

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Author Contributions

B. Albero: investigation, methodology, validation, review, and editing. **P. Sánchez-Argüello:** conceptualization, investigation, methodology, validation, review, and editing. **A. Martín-Esteban:** conceptualization, funding acquisition, project administration, supervision, and writing—review and editing. **E. Tampio:** funding acquisition, resources, and writing—review and editing. **I. Laaksonen:** resources and writing—review and editing. **R. A. Pérez:** conceptualization, inves-

https://doi.org/10.1021/acsenvironau.4c00092
ACS Environ. Au 2025, 5, 376−386

tigation, supervision, writing—original draft preparation, and writing—review and editing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is part of the project "EOM4Soil: External organic matters for climate mitigation and soil health" in the framework of the European Joint Programme for SOIL "Towards climate-smart sustainable management of agricultural soils" (EJP SOIL) funded by the EU Horizon 2020 Research and Innovation Programme (Grant Agreement No. 862695). The authors wish to thank Helsinki Region Environmental Services HSY for providing the sewage sludge and pyrolyzed biochar materials.

■ REFERENCES

(1) Eurostat, E. C. Waste statistics - Statistics Explained. 2020, https://ec.europa.eu/eurostat/statistics-explained/SEPDF/cache/1183.pdf (accessed April 8, 2024).
(2) Alvarenga, P.; Carneiro, J. P.; Fangueiro, D.; Cordovil, C. M. d. S.; Bernal, M. P. Managing Organic Amendments in Agroecosystems to Enhance Soil Carbon Storage and Mitigate Climate Change. In *Climate Change and Soil Interactions*; Elsevier, 2020; pp 89−141.
(3) Buta, M.; Hubeny, J.; Zieliński, W.; Harnisz, M.; Korzeniewska, E. Sewage sludge in agriculture − the effects of selected chemical pollutants and emerging genetic resistance determinants on the quality of soil and crops − a review. *Ecotoxicol. Environ. Saf.* **2021**, *214*, 112070.
(4) Hudcová, H.; Vymazal, J.; Rozkošný, M. Present restrictions of sewage sludge application in agriculture within the European Union. *Soil Water Res.* **2019**, *14*, 104−120.
(5) Regulation 2019/1009. Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products. http://data.europa.eu/eli/reg/2019/1009/oj (accessed May 25, 2023).
(6) Brändli, R. C.; Bucheli, T. D.; Kupper, T.; Furrer, R.; Stahel, W. A.; Stadelmann, F. X.; Tarradellas, J. Organic pollutants in compost and digestate: Part 1. Polychlorinated biphenyls, polycyclic aromatic hydrocarbons and molecular markers. *J. Environ. Monit.* **2007**, *9*, 456−464.
(7) Martín-Pozo, L.; de Alarcón-Gómez, B.; Rodríguez-Gómez, R.; García-Córcoles, M. T.; Çipa, M.; Zafra-Gómez, A. Analytical methods for the determination of emerging contaminants in sewage sludge samples. A review. *Talanta* **2019**, *192*, 508−533.
(8) Pérez, R. A.; Sánchez-Brunete, C.; Albero, B.; Miguel, E.; Tadeo, J. L.; Alonso, J.; Lobo, M. C. Quality assessment of three industry-derived organic amendments for agricultural use. *Compost Sci. Util.* **2016**, *24*, 190−202.
(9) Bonomo, M. M.; Morozesk, M.; Duarte, I. D.; Rocha, L. D.; Fernandes, M. N.; Matsumoto, S. T. Sewage sludge hazardous assessment: chemical evaluation and cytological effects in CHO-k1 cells. *Environ. Sci. Pollut. Res.* **2016**, *23*, 11069−11075.
(10) Pardo, T.; Clemente, R.; Alvarenga, P.; Bernal, M. P. Efficiency of soil organic and inorganic amendments on the remediation of a contaminated mine soil: II. Biological and ecotoxicological evaluation. *Chemosphere* **2014**, *107*, 101−108.
(11) Alvarenga, P.; Mourinha, C.; Farto, M.; Palma, P.; Sengo, J.; Morais, M. C.; Cunha-Queda, C. Ecotoxicological assessment of the potential impact on soil porewater, surface and groundwater from the use of organic wastes as soil amendments. *Ecotoxicol. Environ. Saf.* **2016**, *126*, 102−110.
(12) Escher, B. I.; Stapleton, H. M.; Schymanski, E. L. Tracking complex mixtures of chemicals in our changing environment. *Science* **2020**, *367*, 388−392.
(13) Tampio, E.; Laaksonen, I.; Rimhanen, K.; Honkala, N.; Laakso, J.; Soinne, H.; Rasa, K. Effect of manure co-digestion on methane production, carbon retention, and fertilizer value of digestate. *Sci. Total Environ.* **2024**, *927*, 172083.
(14) Sánchez-Argüello, P.; Franco, D.; Fernández, M. D. Combined cytotoxicity of ZnO nanoparticles and chlorpyrifos in the rainbow trout, *Oncorhynchus mikyss*, gonadal cell line RTG-2. *Aquat. Toxicol.* **2023**, *261*, 106612.
(15) Wluka, A. K.; Huang, Y.; Coenen, L.; Dsikowitzky, L.; Schwarzbauer, J. Structural diversity of organic contaminants in sewage sludge: a comparison of sewage fingerprints from Germany and China. *Discovery Water* **2021**, *1*, 4.
(16) Aznar, R.; Albero, B.; Pérez, R. A.; Sánchez-Brunete, C.; Miguel, E.; Tadeo, J. L. Analysis of emerging organic contaminants in poultry manure by gas chromatography-tandem mass spectrometry. *J. Sep. Sci.* **2018**, *41*, 940−947.
(17) Cai, J.; Zhang, H.; Wu, X.; Liu, Y.; Wu, Y.; Wang, J.; Zhang, C.; Sun, B.; Wu, F. Straw return decreases polycyclic aromatic hydrocarbon (PAH) accumulation in winter wheat and human health risk by enhancing PAH dissipation in rhizosphere soil. *Pedosphere* **2024**, *34* (4), 699−708.
(18) Li, H.; Ma, Y. Field study on the uptake, accumulation, translocation and risk assessment of PAHs in a soil-wheat system with amendments of sewage sludge. *Sci. Total Environ.* **2016**, *560*−*561*, 55−61.
(19) Trapp, S.; Eggen, T. Simulation of the plant uptake of organophosphates and other emerging pollutants for greenhouse experiments and field conditions. *Environ. Sci. Pollut. Res.* **2013**, *20* (6), 4018−4029.
(20) Lao, Z. L.; Wu, D.; Li, H. R.; Feng, Y. F.; Zhang, L. W.; Jiang, X. Y.; Liu, Y. S.; Wu, D. W.; Hu, J. J. Uptake, translocation, and metabolism of organophosphate esters (OPEs) in plants and health perspective for human: A review. *Environ. Res.* **2024**, *249*, 118431.
(21) Hardegen, J.; Braeutigam, P.; Abendroth, C.; Wichard, T. Bisphenol A: Quantification in Complex Matrices and Removal by Anaerobic Sludges. *Pollutants* **2021**, *1*, 194−206.
(22) Golovko, O.; Ahrens, L.; Schelin, J.; Sörengård, M.; Bergstrand, K. J.; Asp, H.; Hultberg, M.; Wiberg, K. Organic micropollutants, heavy metals and pathogens in anaerobic digestate based on food waste. *J. Environ. Manag.* **2022**, *313*, 114997.
(23) Löser, C.; Ulbricht, H.; Seidel, H. Degradation of polycyclic aromatic hydrocarbons (PAHs) in waste wood. *Compost Sci. Util.* **2004**, *12*, 335−341.
(24) Wang, J.; Odinga, E. S.; Zhang, W.; Zhou, X.; Yang, B.; Waigi, M. G.; Gao, Y. Polyaromatic hydrocarbons in biochars and human health risks of food crops grown in biochar-amended soils: A synthesis study. *Environ. Int.* **2019**, *130*, 104899.
(25) Buss, W.; Hilber, I.; Graham, M. C.; Mašek, O. Composition of PAHs in biochar and implications for biochar production. *ACS Sustain. Chem. Eng.* **2022**, *10*, 6755−6765.
(26) Zielińska, A.; Oleszczuk, P. The conversion of sewage sludge into biochar reduces polycyclic aromatic hydrocarbon content and ecotoxicity but increases trace metal content. *Biomass Bioenergy* **2015**, *75*, 235−244.
(27) Zielińska, A.; Oleszczuk, P. Effect of pyrolysis temperatures on freely dissolved polycyclic aromatic hydrocarbon (PAH) concentrations in sewage sludge-derived biochars. *Chemosphere* **2016**, *153*, 68−74.
(28) Alipour, M.; Asadi, H.; Chen, C.; Besalatpour, A. A. Fate of organic pollutants in sewage sludge during thermal treatments: Elimination of PCBs, PAHs, and PPCPs. *Fuel* **2022**, *319*, 123864.
(29) Corrales, J.; Kristofco, L. A.; Steele, W. B.; Yates, B. S.; Breed, C. S.; Williams, E. S.; Brooks, B. W. Global assessment of Bisphenol A in the environment: Review and analysis of its occurrence and bioaccumulation. *Dose Response* **2015**, *13*, 1559325815598308.
(30) Torres-García, J. L.; Ahuactzin-Pérez, M.; Fernández, F. J.; Cortés-Espinosa, D. V. Bisphenol A in the environment and recent advances in biodegradation by fungi. *Chemosphere* **2022**, *303*, 134940.

(31) Kumagai, S.; Ono, S.; Yokoyama, S.; Kameda, T.; Yoshioka, T. Fate of bisphenol A pyrolysates at low pyrolytic temperatures. *J. Anal. Appl. Pyrolysis* **2017**, *125*, 193−200.
(32) Sarvi, M.; Kainulainen, A.; Malk, V.; Kaseva, J.; Rasa, K. Industrial pilot scale slow pyrolysis reduces the content of organic contaminants in sewage sludge. *Waste Manag.* **2023**, *171*, 95−104.
(33) Adedigba, B. M.; Semple, K. T. Bioavailability of Persistent Organic Pollutants in Soils: Concept, Analytical Tools, and Application. *Compr. Anal. Chem.* **2015**, *67*, 493−512.
(34) Pérez, R. A.; Albero, B.; Tadeo, J. L.; Fraile, M. V.; Sánchez-Brunete, C. Determination of PAHs in soil leachates by magnetic solid-phase extraction using nanoparticles and gas chromatography-tandem mass spectrometry. *Anal. Methods* **2014**, *6*, 1941−1950.
(35) Pérez, R. A.; Albero, B.; Tadeo, J. L.; Molero, E.; Sánchez-Brunete, C. Application of magnetic iron oxide nanoparticles for the analysis of PCBs in water and soil leachates by gas chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2015**, *407*, 1913−1924.
(36) Cristale, J.; Álvarez-Martín, A.; Rodríguez-Cruz, S.; Sánchez-Martín, M. J.; Lacorte, S. Sorption and desorption of organophosphate esters with different hydrophobicity by soils. *Environ. Sci. Pollut. Res.* **2017**, *24*, 27870−27878.
(37) Roques, O.; Bedell, J. P.; Le Maux, J.; Wiest, L.; Nélieu, S.; Mougin, C.; Cheviron, N.; Santini, O.; Lecomte, V.; Bayard, R. Assessing the potential ecotoxicological risk of different organic amendments used in agriculture: approach using acute toxicity tests on plants and earthworms. *Arch. Environ. Contam. Toxicol.* **2023**, *84*, 248−266.
(38) Bloem, E.; Albihn, A.; Elving, J.; Hermann, L.; Lehmann, L.; Sarvi, M.; Schaaf, T.; Schick, J.; Turtola, E.; Ylivainio, K. Contamination of organic nutrient sources with potentially toxic elements, antibiotics and pathogen microorganisms in relation to P fertilizer potential and treatment options for the production of sustainable fertilizers: A review. *Sci. Total Environ.* **2017**, *607−608*, 225−242.
(39) Han, H.; Buss, W.; Zheng, Y.; Song, P.; Khalid Rafiq, M.; Liu, P.; Mašek, O.; Li, X. Contaminants in biochar and suggested mitigation measures − a review. *Chem. Eng. J.* **2022**, *429*, 132287.
(40) Zhang, J.; Wang, M.; Yin, C.; Dogot, T. The potential of dairy manure and sewage management pathways towards a circular economy: A meta-analysis from the life cycle perspective. *Sci. Total Environ.* **2021**, *779*, 146396.
(41) Merdun, H.; Laouge, Z. B.; Sezgin, I. V.; Ciggin, A. S. Effects of biological pre-digestion of sewage sludge processed by fast pyrolysis on bio-oil yield and biochar toxicity. *Waste Manag.* **2023**, *157*, 149−158.
(42) Hoang, S. A.; Bolan, N.; Madhubashani, A. M. P.; Vithanage, M.; Perera, V.; Wijesekara, H.; Wang, H.; Srivastava, P.; Kirkham, M. B.; Mickan, B. S.; Rinklebe, J.; Siddique, K. H. M. Treatment processes to eliminate potential environmental hazards and restore agronomic value of sewage sludge: A review. *Environ. Pollut.* **2022**, *293*, 118564.

https://doi.org/10.1021/acsenvironau.4c00092
ACS Environ. Au 2025, 5, 376−386