Synergetic Effect of Digestate Dissolved Organic Matter and Phosphogypsum Properties on Heavy Metals Immobilization in Soils

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Abstract. The main idea was to justify the natural, technological, and ecological aspects of digestate-based composite for heavy metals (HMs) binding in soil due to organic matter content and mineral additives' biosorption properties. The study aimed to determine the potential of a composite made from digestate and phosphogypsum for remediation of HMs polluted soils and the role of dissolved organic matter (DOM) in binding HMs. Methods used included a literature review to identify the mechanisms for HM binding to digestate DOM, a laboratory setup for producing a digestate-based composite with digestate (from manure or sewage sludge) mixed with phosphogypsum, and an analysis of digestate fluorescence properties. Results show that a composite based on digestate from manure as feedstock had a higher fluorescence complexity index than a composite with sewage sludge digestate (2.2 and 1.71, respectively). However, the DOM stability in the sewage sludge digestate composite was higher than reported in the literature, probably due to the mineral composition of phosphogypsum, which resulted in a high HMs sorption capacity and its positive effect on soil microbial activity. Based on the theoretical substantiation of DOM content and its binding properties, manure was the most effective feedstock type out of the two tested if digestate was used for HM remediation. Using a digestate-based composite with phosphogypsum can potentially reduce the ecological risk levels imposed by HM-contaminated soils from considerably too low.

Keywords: anaerobic digestion, chemical pollution, ecological risk, fertilizer, soil degradation, soil restoration.

1 Introduction

Due to rapid industrial growth, the accumulation of heavy metals (HMs) in agricultural soils poses severe risks for humans as the metals may be ingested if taken up by plants and further transported along the food chain [1]. The most predominant sources of soil contamination with HMs are as follows: mining industries, e.g., coal mining [2] and non-ferrous metal mining processes [3] through migration from waste dumps [4] and atmospheric deposition pathway [5], direct mineral phosphorus fertilizers application [6], that contributes to significant cadmium and uranium accumulation in the soil [7], and organic fertilizers (mostly manure) application [8]. HMs potentially contained in manure-based fertilizers might accumulate in soils above the ecologically safe threshold levels, causing ecological risks [9]. Human health risks increase if the HMs are bioavailable for plant uptake and if they leach from soil to surface- or groundwater. The latter mostly depends on the chemical structure of soil compounds that may bind to HMs, the strength of the bindings, and environmental conditions such as pH; high acidity increases the mobility of many HMs [10].

Various soil remediation mechanisms play a key role in alleviating those risks and possibly reaching environmental goals, such as the EU soil strategy for 2030 [11]. These mechanisms are based on physical and chemical processes: precipitation, oxidation, ion exchange, nanofiltration, osmosis, and adsorption [12], but some of the methods could be combined with biological processes in soil to increase their efficiency.

The most effective in-situ bioremediation approach focuses on changing soil parameters (e.g., acidity, alkalinity, cation exchange capacity) and immobilizing heavy metals in durable chemical materials through biosorption, bioprecipitation, and fixation or complexation. In this context, a fertilization strategy based on HM-free fertilizers could prevent soil pollution and remediate already polluted sites if compounds in the fertilizers bind HMs. Digestate from anaerobic digestion (AD) of organic residues is currently widely used as a biofertilizer in the EU [13], but in Ukraine, this is a relatively rare practice [14]. Besides the positive effect on crop yield and soil properties [15], digestate contains soluble organic matter essential in binding HMs. When applying digestate to soils, the dissolved organic matter (DOM) affects the mobility of HMs, which could potentially contribute to the restoration of soil resources [16].

In this context, using digestate from AD can be a promising strategy to reduce the concentration of bioavailable HMs in soils [17]. At the same time, nutrients in the digestate can substitute part of the chemical fertilizers used to enhance crop yields.

Digestate can also be converted into value-added products such as biochar, activated carbon, or composite materials that are effective adsorbents capable of remediating soils contaminated with HMs [18]. Biochar can have multiple positive effects on soils, e.g., immobilization of HMs ions, carbon storage, stimulation of soil microbial activity, and improvement of soil fertility and quality [19].

DOM can be used for bioleaching of HMs from soils due to carboxyl, hydroxyl, phenolic, and sulfhydryl groups, which can form metal complexes [20]. Besides factors such as metal type and environmental conditions, the presence of appropriate molecular groups in DOM is the main factor affecting the binding properties of DOM-HMs [21].

Alternatively, a way to increase the potential for digestate to bind HMs would be to produce new biocomposite materials by mixing with inorganic compounds, such as phosphogypsum.

2 Literature Review

A literature review was performed to define the role of DOM in digestate on binding HMs and indicate knowledge gaps. For this, a literature search in Scopus was done for articles published in the period 2013–2023, using the keyword string [("dissolved organic matter" OR "DOM") AND ("heavy metal*" OR "HM*") AND digestate]. This resulted in 798 articles from different research areas. VOSviewer software (version 1.6.15) was used to construct and visualize co-occurrence networks of the keywords extracted from those papers (Figure 1). This resulted in keyword clusters describing four research areas:

– blue cluster – fluorescence properties of DOM, chemical characteristics, and interactions with HM;
– red cluster – biogeochemical studies of soil and water pollution with heavy metals and soil organic matter;
– green cluster – using infrared spectroscopy for analysis of complex organic substances;
– purple cluster – studies of interactions between DOM, particularly humic substances, and heavy metals using, e.g., parallel factor analysis.
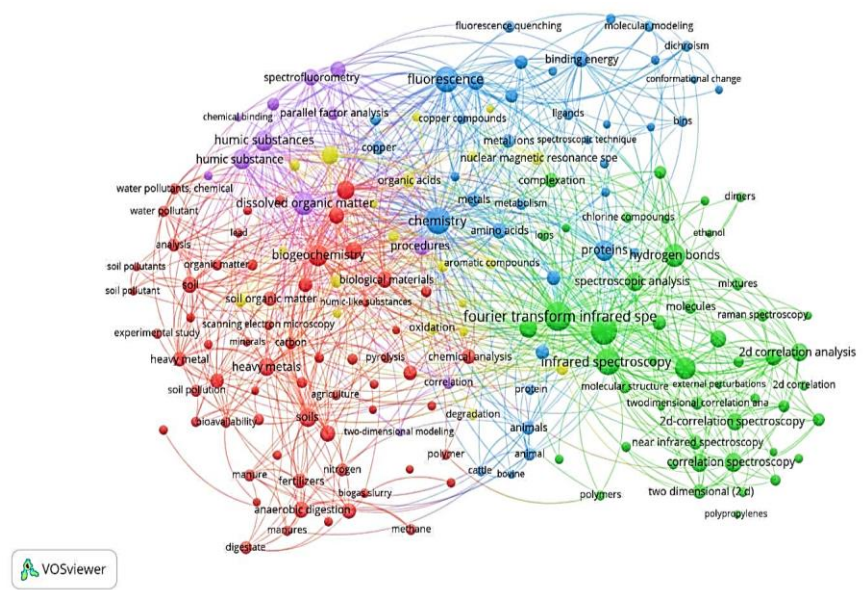

Figure 1 – Co-occurrence network of keywords extracted from 798 papers about DOM and HM papers published in Scopus 2013–2023 (only keywords appearing with a frequency > 5)

There are 3 main fluorescent components in DOM (humic-like, fulvic-like, and protein-like substances) that vary concerning how strongly they bind to specific heavy metal ions [22]. According to Huang et al. [23], humic-like substances had higher conditional stability constant $\log(K_M)$ values for DOM-Cu^{2+} than fulvic acid-like substances, followed by protein-like substances. This suggests a higher stability for complexes of Cu with humic substances than with other fluorescence components. The same authors also performed experiments with Zn^{2+} ions and obtained similar results.

Guo et al. [16] studied the effect of DOM on Cr^{3+}, Cu^{2+}, and Fe^{3+} in soil and found that fluorescent components in digestate DOM can interact with HMs on the soil surface and form a stable chemical compound that influence the mobility, bioavailability, and transformation of those HMs. Based on those results, the researchers could show that bindings between Cr^{3+} and fulvic-like components had the highest binding stability and the highest total ligand concentration. However, the combination of fulvic-like and protein-like substances resulted in the lowest $\log(K_M)$ value, indicating that the binding stability of the Cr^{3+} ion decreased when combined with tryptophan-like and fulvic-like substances [16].

The $\log(K_M)$ values were 5.05–5.29 for fluorescent components in the DOM-Cu^{2+} complexes. The fulvic-like components also had a relatively higher $\log(K_M)$ value than the other identified components, revealing a relatively stable complexation. The combination of fluorescence peaks for a protein-like and a fulvic-like substance presented a weak binding stability of Cu^{2+} ion in the DOM-Cu^{2+} complexes [16].

Digestate DOM can form highly stable complexes with Fe^{3+} ions irrespective of the organic component. For all three DOM components, Guo et al. found that DOM-Pb^{2+} had the lowest $\log(K_M)$ among the HMs studied [16]. Moreover, Yu et al. [24] showed that the binding strength for Al^{3+} identified as the conditional stability constant depends on fertilization strategy, meaning the source of DOM. They found that the binding stability of Al^{3+} with humic-like substances was lower when the soil was treated with mineral fertilizer compared to control samples with no fertilization, but for control samples, the stability was higher for humic-like substances than for fulvic-like substances.

The parameter $\log(K_M)$ is used when evaluating the pH and ionic strength (IS) dependent binding heterogeneities. Specifically, the $\log(K_M)$ parameter for tryptophan-like and humic-like components was higher at pH 6.0 than at pH 4.7 [25]. The same study demonstrated no apparent effect of IS on the $\log(KM)$ values.

Additionally, humic acids and amino acids contained in DOM are bio-stimulants for plant growth, which makes digestate from AD a potential biofertilizer due to its high organic matter content. Compared to control samples, the positive growth effect on plants cultivated with DOM has been confirmed [26] and was attributed to amino compounds and humic substances.

There are specific differences at the molecular level in the binding properties of different types of DOM and heavy metals such as Cd and Cu. The binding mechanisms between Cd, Cu, and DOM from different sources have been studied [27, 28]. The study focused on three different sources of DOM: rice straw, compost from a mix of straw and chicken manure, and biochar from rice straw. The binding affinity of Cd and Cu with DOM functional groups was shown to differ within one substrate type and for one metal (Cd or Cu) between DOM derived from the different sources. That is also in line with the results by Yu et al. (2012) for Al (III) [24].

The differences in binding characteristics of Cd^{2+} with DOM derived from different sources were identified as follows (Figure 2):

a) for compost from straw and chicken manure, the binding affinity dropped in the order phenolic groups > carboxyl groups;

b) for rice straw, the binding affinity dropped the order of carbonyl groups of proteins > phenolic groups > carboxyl groups > the C = C stretching of aromatic groups [28];

c) for biochar from rice straw, the binding affinity dropped in the order of phenolic groups and carboxyl groups > polysaccharide and the N = O stretching of aromatic groups > aliphatic groups > the C = C stretching of aromatic groups [27].

For Cu^{2+}, the differences in binding affinity could be described as follows (Figure 2):

a) for compost from straw and chicken manure, the binding affinity dropped in the order carboxyl groups > phenolic groups;

b) for rice straw, the binding affinity dropped in the order phenolic groups > aliphatic groups > carboxyl groups > the C = C stretching of aromatic groups > carbonyl groups of proteins [28];

c) for biochar from rice straw, the binding affinity dropped in the order polysaccharides > N = O stretching of aromatic groups and aliphatic groups > phenolic groups > carboxyl groups [27].

In summary, phenolic groups had a higher potential to bind with Cd^{2+} than with Cu^{2+} for anaerobically or aerobically treated organic material. Aliphatic groups and the C = C stretching of aromatic groups showed the lowest binding potential irrespective of the origin of DOM and the specific HM. Carboxyl groups had higher binding affinity to Cu^{2+} than to Cd^{2+} in DOM derived from compost and rice straw, but the reverse was observed in DOM derived from biochar. These results stress the importance of investigating the HM binding potential of specific organic matter sources to identify the best type for binding HMs in soils.
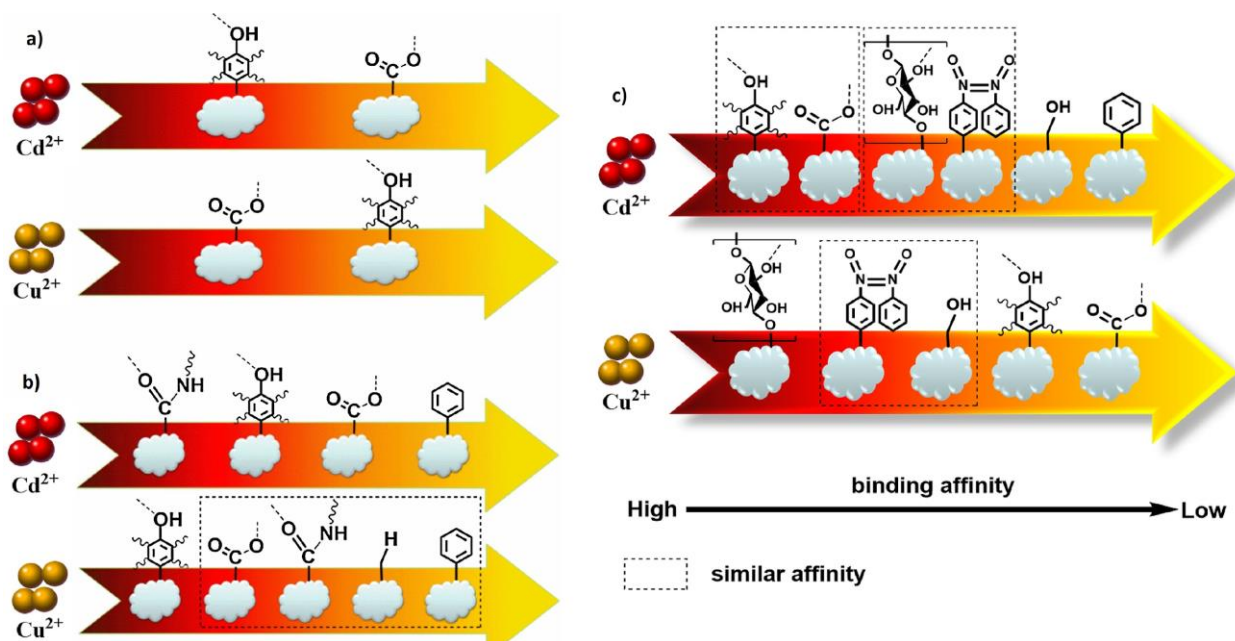
Figure 2 – Mechanisms of Cd and Cu complexation with DOM derived from different sources [27, 28]:
a – compost from straw and chicken manure; b – rice straw; c – biochar from rice straw

Moreover, the composition of DOM in soils is dynamic, as organic matter is degraded and transformed, which can significantly alter the binding of HMs. Understanding these processes is crucial for effectively implementing methods for HM immobilization. Wang et al. [29] investigated DOM transformation during composting of municipal solid waste and determined that the binding of Cu and Al with DOM mainly occurred during the anabolism of the humic fraction. In contrast, Fe, Mn, Co, Zn, Ni, Sr, and Cr binding occurred because of the small molecular size of humic matter synthesis. This study also established the dominant groups of microorganisms responsible for DOM degradation and the humification process.

A recent study by Cattin et al. [30] showed that organic carbon in digestate, especially the solid fraction, is highly available for soil microorganisms and led to an increased soil microbial community and higher carbon use efficiency. Similarly, Doyeni et al. [31] found that manure-based digestate positively influenced soil microbial activity and the soil's humification degree of organic matter. However, there is a lack of studies on the relationship between digestate as a biofertilizer, soil microbial activity, and the impact on long-term binding of HMs.

Based on this literature review, we suggest that immobilization of heavy metals through binding with DOM functional groups, for example, in digestate, will not be a long-term process due to continuous soil organic matter turnover and humification processes. There seems to be scope for increasing the HM binding capacity using different additives for soil remediation purposes. From a sustainability perspective, suitable additives to biofertilizers from anaerobic digestion contain nutrients, are by-products from some production processes, and

should be reused. A previous study suggests a considerable potential to use phosphogypsum for this purpose [32]. Phosphogypsum in a biocomposite mix with digestate can influence the binding of HMs bindings in several ways.

Like other sorption methods, e.g., applied for Cr adsorption [33] with bentonite clays [34], phosphogypsum has good prospects in HMs fixation and raw material recycling. Moreover, surface modification can significantly improve the adsorption properties of phosphogypsum. In the study [35], phosphogypsum was modified with sodium dodecyl benzene sulfonate, and the adsorbent showed the ability to take Cu^{2+}.

A solidification/stabilization technique for soil remediation involves the removal of heavy metals from the soil by fixing the pollutants in a structured matrix or making them insoluble. One such study [36] involved using a binder made from a mixture of slag, phosphogypsum, and lime. The results showed an effective fixation of Pb using this binder, suggesting that this mixture can be used for the remediation of soils contaminated with Pb.

However, there is a lack of studies that systematically assess the potential for bioremediation of soils polluted with HM using bio-composites, including their efficiency and ecological safety.

Therefore, this article's main aim was to determine the bioremediation potential of a composite from digestate and phosphogypsum for mitigating heavy metals in polluted soils and the role of the DOM component in binding the HMs.

The following tasks were performed to accomplish it:

1) to develop a comprehensive model for heavy metals immobilization in soils mixed with the digestate/ phosphogypsum composite;

2) to predict theoretically the type of organic substrate for anaerobic digestion that would result in a digestate with the most effective soil HMs binding based on DOM characteristics;

3) to verify experimentally the positive effect of phosphogypsum addition to digestate on HMs binding in soils.

3 Research Methodology

3.1 Sample analysis and data processing

We sampled digestate from batch experiment biogas reactors (2 L) fed with chicken manure from a local chicken farm and sewage sludge from a wastewater treatment plant in Sumy Region, Ukraine. At the beginning of incubation, 15 % (dry matter mass) of phosphogypsum was added from a phosphogypsum dump in Sumy. Anaerobic digestion was run for 30 days under mesophilic conditions (process temperature was around 38 °C). Three replicate samples were incubated for each substrate type to ensure representative results. After the incubation, it was centrifuged (10 000 rpm for 10 min), filtered (0.45 μm membrane filter), and stored at 4 °C for further chemical analysis. Total solids, ratio of volatile solids to total solids (VS/TS), pH, and dissolved organic carbon (DOC) were analyzed according to standard methods [37]. A Portable Total Organic Carbon Analyzer 820 w/ 800 Automatic Sampler was used for DOC.

Elemental analyses were done using the X-ray fluorescence method (XRF) using the energy-dispersive X-ray fluorescence spectrometer ElvaX Light SDD. The phase composition of phosphogypsum samples was determined by X-ray diffraction (automatic diffractometer DRON-4-07, Burevisnyk NPP, Ukraine).

To analyze DOM in digestates, we used a fluorometer to obtain fluorescence data and calculated the fluorescence and absorbance indices using the R software. Parallel component analysis (PARAFAC) was done in MATLAB to identify different components from the fluorescence signals (humic-like, fulvic-like, and protein-like substances) and calculate their maximum intensities.

We calculated the modified fluorescence complexity index (FCI) to represent DOM stability, following Muller et al. [38], who proposed to use a ratio of the sum of a fluorescence zone volume related to humic-like and fulvic-like components to the fluorescence zone volume related to protein-like components. The present study used maximum intensities of fluorescence components instead of fluorescence zone volume. Thus, the modified fluorescence complexity index FCI^m is as follows:

$$FCI^m = \frac{F_{max}(HLA) + F_{max}(FLA)}{F_{max}(PLC)}, \qquad (1)$$

where $F_{max}(X)$ is the maximum intensity function of humic-like acids (HLA), fulvic-like acids (FLA), and protein-like components (PLC).

3.2 Mechanisms for heavy metals fixation

The binding parameters of the DOM-HMs could be determined with a non-linear equation derived by Ryan and Weber [39]. The Ryan-Weber equation assumes a 1:1 ratio between ligands and metal ions.

To estimate the detailed binding properties of various organic components, we applied the modified Stern-Volmer equation:

$$\frac{F_0}{F_0 - F} = \frac{1}{f \cdot K_M} + \frac{1}{f}, \qquad (2)$$

where F, F_0 – the measured DOM abundances (here, the fluorescent component intensity) at a metal concentration of C_M and the beginning of titration before metal addition, respectively; f – parameter representing the fraction of the initial abundance of DOM components corresponding to metal binding; K_M – the conditional stability constant [40].

According to Molaey et al. [20], carboxylate functional groups of the volatile fatty acids (VFAs) can coordinate monodentate with divalent metal cations. Based on this assumption, the functional mechanism for HMs complexation with digestate DOM in soils was described (Figure 3).
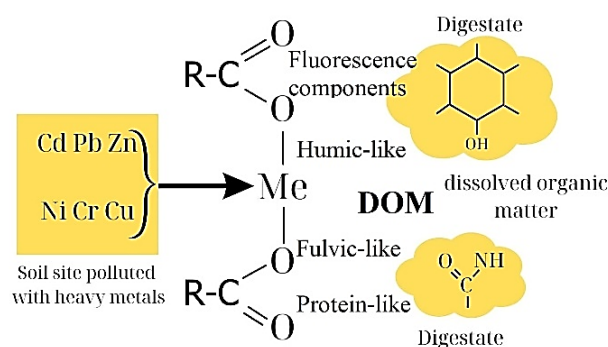

Figure 3 – Effect of digestate dissolved organic matter on heavy metals complexation in polluted soil

Dissolved organic matter may also increase the dissolution of potentially toxic metals because of complexation. Complexing groups, e.g., carboxylate in the DOM, may enhance the dissolution of potentially toxic metals [41].

3.3 Risk assessment

Ali and Muhammad [42] and Zhou et al. [43] proposed a method to assess ecological risks caused by soil pollution with HMs using a potential ecological risk index (ER). This is calculated as:

$$ER = \sum_{i=1}^{n} \frac{T_r^i \cdot C_i}{C_b}, \qquad (3)$$

where ER – the potential ecological risk index; T_r^i – toxic response factor for i[th] HM; C_i is the concentration of i-th HM in soil; and C_b – the background concentration of i-th HM in soil. The resulting ER values can then be used to assess the risk (Table 1) [43].

Table 1 – Classification of potential ecological risk (ER)

Value of ER index	Degree of ER
ER <40	Low
$40 \leq ER < 80$	Moderate
$80 \leq ER < 160$	Considerable
$160 \leq ER < 320$	Serious
ER ≥ 230	Severe pollution

An agricultural area in the Sumy Region, Ukraine, where soils were polluted after military bombing in 2022-2023, was used for ecological risk analysis and to test the effectiveness of the proposed bio-composite for heavy metals immobilization and reduction of the risk index. The soils had elevated levels of Zn, Cu, and Pb (Table 1).

Table 2 –Heavy metal concentrations in agricultural soils in Sumy, exposed to bombings in 2022-2023, pre-bombing values, and toxic response factors

Heavy metal	Background value C_b, mg/kg	Concentration C_i, mg/kg	Toxic response factor T_r^i [42]
Zn	67.07	261.61	1
Ni	6.28	7.44	5
Cu	36.24	165.43	5
Cd	0.52	0.76	30
Pb	20.63	110.88	5

4 Results

4.1 A systematic approach to heavy metals immobilization using digestate-based composite

Based on the theoretical modeling, the proposed biocomposite made from digestate and phosphogypsum could be very effective for long-term HMs fixation, with several processes involved (Figure 4).

Chemical binding of HMs with digestate DOM according to the presence of three main fluorescence components (fulvic-, humic-, and protein-like) is different for particular HMs. We used the main results from the literature review to model the complex process of HMs immobilization to DOM:

1) humic-like substances have the best binding properties with Cu^{2+} and Zn^{2+} ions;

2) fulvic-like components have the highest binding stability for Cr^{3+} and Pb^{2+} ions;

3) a combination of fulvic-like and protein-like substances decreases the binding stability of any HMs ions;

4) stable complexes are formed with Fe^{3+} ions irrespective of the organic component;

5) the conditional stability constant for protein-like and humic-like bindings with HMs is higher at a pH close to neutral than at acidic pH values.
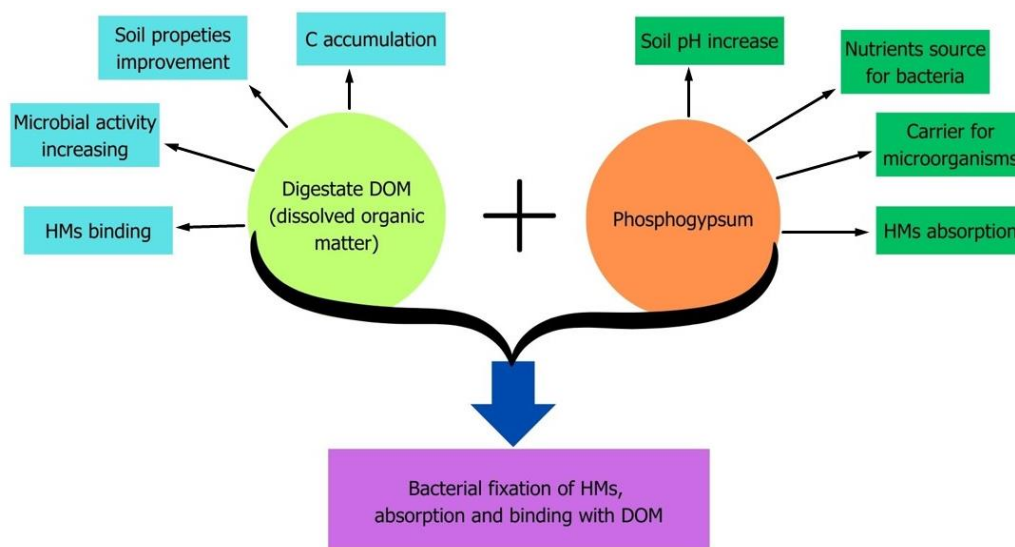

Figure 4 – Scheme for modeling the synergetic effects of digestate and phosphogypsum on immobilization of HMs in soil through biochemical mechanisms

The content of DOM in digestate depends primarily on the feedstock type and stage of the anaerobic digestion. The most common feedstocks used for anaerobic digestion today can be divided into the following groups: manure (chicken, pig, and cow), agricultural residues and energy crops, food waste, and sewage sludge from wastewater plants. Research [44] has shown that co-digestion of manure, e.g., chicken manure, with plant residues can increase biogas production and potentially be applied on an industrial scale.

Different feedstock types have different chemical and physical characteristics, DOM content, and ratios of fulvic-, humic- and protein-like substances. Digestate from each substrate type usually includes all three fluorescence compounds with different ratios representing indexes [45]. During digestion, the amount of each fluorescence compound changes and continues to change after the digestate is applied to the soil.

In this sense, it is essential to evaluate the DOM stability using FCI, reflecting the proportion of fulvic-like

and humic-like substances to protein-like substances. This index will characterize the ability of digestate as a biofertilizer to bind HMs in the soil.

The role of soil microbial activity for HM immobilization is explained mainly by microbial absorption and microbially induced precipitation of HMs in the soil. As was shown before, digestate has a positive effect on soil parameters and microbial activity. Therefore, the immobilization of HMs in soils is also related to biochemical processes. Reactions such as HMs complexation with DOM are possible when the environmental conditions are favorable, e.g., access to organic matter-, macro- and micro-nutrients needed for microbial growth. Urease-producing bacteria are primarily responsible for microbial sorption and precipitation of HMs, particularly Cd, Cu, and Zn [46]. That is why microbial-induced precipitation plays a crucial role in HMs reduction in the soil.

Growth conditions for soil microbiota could be improved by adding phosphogypsum to digestate. The nutrients in phosphogypsum positively affect the vital activity of bacteria involved in the decomposition and humification processes [47]. The toxic effects of certain elements, such as aluminum and manganese, can inhibit the growth of soil microbes involved in the decomposition of organic residues with the subsequent formation of high molecular weight compounds or humus substances. As an additive, phosphogypsum increases the availability of nutrients that are resources for plant and microbial growth.

Phosphogypsum also affects the immobilization of heavy metals due to its structure, which can neutralize possible inhibitory effects of HMs on soil microbes. This is very important for long-term DOM degradation and transformation. Agriculture could become the main consumer of phosphogypsum as a fertilizer, as it is enriched with calcium, phosphorus, and sulfate. That helps improve yields and soil properties (e.g., restoration of acidic soils and increased moisture retention).

4.2 Fluorescence complexity index as a representer of DOM stability

According to our previous study [48], the preferred feedstocks for anaerobic digestion in Ukraine are crops or agricultural residues and animal manure (cattle, pig, chicken) due to those substrates' availability and the agricultural sector's development. The study also indicated that sewage sludge accumulation from wastewater treatment is a growing problem in Ukraine with low utilization in anaerobic digestion, whereas in European countries, sewage sludge is a common feedstock. Thus, we chose manure and sewage sludge for our experiments and mixed them with phosphogypsum before digestion.

In the chicken manure digestate produced after the 30th day batch of anaerobic digestion, both the VS and the DOC were higher than in the sewage sludge digestate, and the DOC was about 20 times higher (Table 3).

Table 3 – pH and concentrations of TS, VS, and DOC in digestate samples resulting from digestion of chicken manure and sewage sludge

Chemical parameter	Sample code (substrate type)	
	CM (Chicken manure)	SS (Sewage sludge)
Parameter value ± standard deviation		
pH	7.25±0.01	7.8±0.02
TS, %	7.00±0.00	3.00±0.00
VS, % of TS	73.3±0.05	64.5±0.04
DOC, mg/L	2086±59.7	112±5.23

The maximum intensities of three components of DOM were calculated based on the PARAFAC analysis and were higher for all three groups in the chicken manure-based digestate (Figure 5), though the difference was not significant for the humic-like and protein-like components.
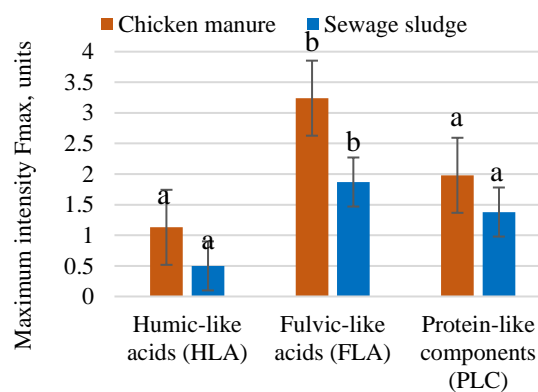

Figure 5 – Mean values (with s.d.) of maximum intensities for fluorescence components (humic-, fulvic-, and protein-like substances) of digestate after anaerobic digestion of chicken manure and sewage sludge

The modified fluorescence complexity index FCI^m (1) was 2.2 in chicken manure digestate and 1.7 in sewage sludge digestate.

The mineral composition of the biocomposite made from sewage sludge and phosphogypsum includes silicon oxide SiO_2, gypsum $CaSO_4 \cdot 2H_2O$, calcite $CaCO_3$, potassium hydroxide KOH, and mascagnite $(NH_4)_2SO_4$, brushite $CaHPO_4 \cdot 2H_2O$, phosphorus oxide P_2O_5, and potassium hydrogen phosphate hydrate $H_5K_2O_6P$ (Figure 6).

It should be noted that there is a biochemical transformation of the treated waste's mineral component, which requires additional research to specify the dose of bio-composites in addition to soils.

The potential ecological risk index was estimated according to equation (3) using initial data from Table 1 and resulted in $ER = 103$, which implies that this area is prone to a considerable ecological risk (Table 2).
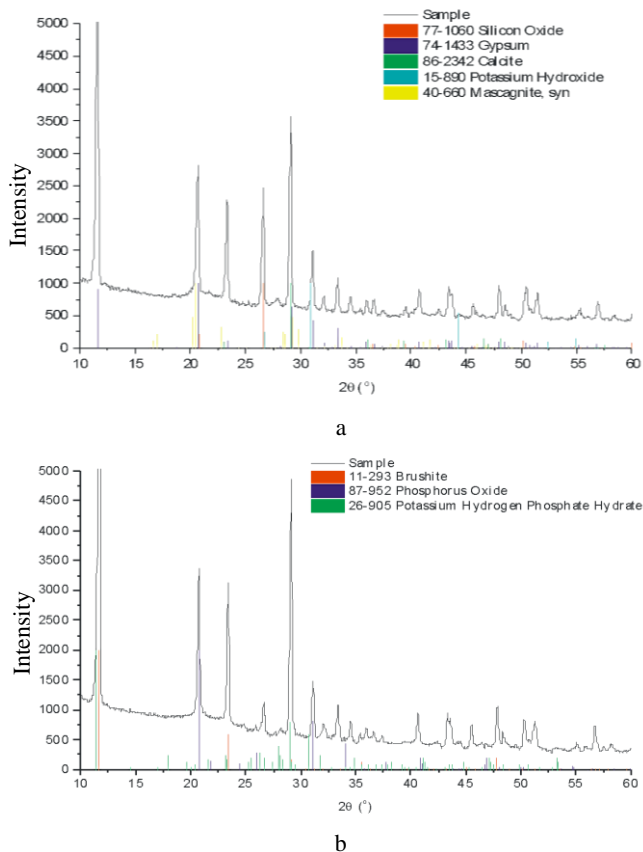
Figure 6 – Results of diffractometric analysis of biocomposite samples based on sewage sludge mixed with phosphogypsum (15 % of mass): a – silicon oxide, gypsum, calcite, potassium hydroxide, and mascagnite; b – brushite, phosphorus oxide, potassium hydrogen, and phosphate hydrate

5 Discussion

According to the findings regarding the affinity of fluorescence components to specific HM and the binding strengths, digestate from chicken manure and sewage sludge likely have different binding properties. Digestate from chicken manure has the potential to bind Cu and Zn, but the binding of Cr and Pb could be lower than for sewage sludge digestate because of the high intensity of fulvic-like and protein-like substances (Figure 5).

On the other hand, digestate from sewage sludge must effectively bind Fe and Al. Moreover, a previous analysis [49] indicated that protein-like substances are predominant in DOM-HMs binding because of higher total ligand concentration. This finding identifies the best substrate for anaerobic digestion with DOM characteristics that can increase HMs complexation. As mentioned, the higher maximum intensity of protein-like substances in manure digestate suggests it has a higher HMs binding potential than sewage sludge digestate.

A recent study [50] showed that manure-based digestate had the highest value of FCI and increased after anaerobic digestion to 0.81–3.20, corresponding to our results of 2.2 for chicken manure digestate. However, the FCI value 1.71 for sewage sludge digestate exceeds previously reported

values of 0.45–1.34 [50]. This could be related to the initial chemical content of the sewage sludge, process temperature, and possibly the microbial activity in the biogas reactor responding to the addition of phosphogypsum. The higher value of FCI indicates a more stable DOM and better-suggesting conditions for binding to HMs.

According to Siebielec et al. [51], digestate from sewage sludge positively affects all soil enzymes (acid and alkaline phosphatases and dehydrogenases), and soil organic carbon contributes to increased soil pH values. The latter would decrease the mobility of HMs in soil.

In summary, the results from this article and the literature review point out that digestate from manure as the main feedstock must be better than sewage sludge digestate when it comes to immobilizing HMs in soils.

The chemical composition of phosphogypsum and its effect on soil properties and possibly on microbial activity, as well as the potential for HMs absorption, indicate that there is a positive synergetic effect from adding phosphogypsum to digestate and form a composite to be used for immobilizing HMs in polluted soils. This aligns with the results obtained by Watson et al. [52], who showed that hydrocar had more sites for partial metal detoxification and colonization by soil microorganisms and thus could be used for soil remediation. Thus, using phosphogypsum in combination with organic components, including digestate, can be a sustainable environmental protection technology using by-products from phosphoric acid and biogas production. The biocomposite can effectively improve soil quality and potentially immobilize heavy metals.

However, more studies are needed to better define the composition of the composite, e.g. in terms of dose of phosphogypsum and organic component to be used. For example, recent results [53] suggested that 1–10 % of phosphogypsum in the composite positively affects soil properties, lower than the 15 % used in the current study. Furthermore, there is scope for more studies to develop and test the technical setup for using bio-composite to clean soils from HMs, as suggested in the author's previous work [54]. Based on the results, we suggest that a digestate-based composite with phosphogypsum could decrease the ecological risk associated with contaminated soils from "considerable" to "low".

More studies are also needed on the possible role of phosphogypsum as a surface for microbial growth in the anaerobic digestion process. Using a supportive growth medium (bio-carrier) for microorganisms is a method that has become widely used in various environmental technologies. The prime function of the bio-carrier is to provide a surface for biofilm development, including the formation of extracellular polymeric substances as a matrix that protects microorganisms from adverse effects of metabolites and inhibitors that cannot penetrate the matrix. Modifications of the carriers include materials that can provide microbes with, e.g., trace elements to facilitate the removal of metabolites or with electron donors.

Another essential criterion for selecting the media is cost. Combined with the value and reusability of by-

products, this leads to a search for cheap and potentially accessible media. In this context, one of our previous studies has suggested using phosphogypsum-based composites for bioremediation of land contaminated with radioactive compounds [55].

Finally, the implementation of the developed biosorption technology has the potential to contribute to achieving several sustainable development goals (SDGs). Using anaerobic digestate from biogas production can contribute to the most significant SDGs challenges:

1) end hunger, achieve food security and improved nutrition, and promote sustainable agriculture (Goal 2);

2) ensure access to affordable, reliable, sustainable and modern energy for all (Goal 7);

3) make cities and human settlements inclusive, safe, resilient and sustainable (Goal 11);

4) take urgent action to combat climate change and its impacts (Goal 13);

5) protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss (Goal 15).

6 Conclusions

The mechanisms for heavy metal binding to DOM were analyzed in a literature review, and the main factors that affect the HM binding properties of digestate-based products were identified.

State-of-the-art states that humic-like substances have the best binding properties with Cu^{2+} and Zn^{2+} ions; fulvic-like components have the highest binding stability for Cr^{3+} and Pb^{2+} ions; a combination of fulvic-like and protein-like substances decreases the binding stability for all HMs ions; stable DOM complexes with Fe^{3+} ions are formed irrespective of the dominant organic components; the conditional stability constant for protein-like and humic-like components is higher at pH close to neutral than for acidic pH.

Theoretical modeling and analyses of fluorescence components and the organic functional groups that bind with heavy metals suggest that manure would be a better feedstock for anaerobic digestion than sewage sludge if the aim is to produce a digestate with a high level of humic substances and good HM binding properties.

Adding 15 mass% (by dry matter) of phosphogypsum to the main feedstock during anaerobic digestion seemed to increase the nutrient availability and stimulate the growth of microorganisms with appropriate enzymatic systems that participate in the humification, and microbial heavy metals fixation processes will be proved in the further study. At the same time, the mineral composition of the digestate-based composite with phosphogypsum shows good sorption properties and would affect the immobilization of heavy metals into its structure.

Further studies will focus on practical and economic evaluations of technological solutions for the biosorption of heavy metals from different types of soils.

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