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# THE USE OF BIOINDICATION TO DETERMINE THE POSSIBILITY OF SLUDGE RECOVERY AFTER BIOLOGICAL TREATMENT OF WASTEWATER

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Abstract. These studies were conducted to test the hypothesis that sewage sludge from Lviv municipal wastewater treatment plants (LMWWTP) facilities can be used to create a substrate that can be used for biological reclamation. The qualitative composition of sewage sludge from Lviv municipal wastewater treatment plants is determined. The study of bioindication of a mixture of sludge with dark gray soil was carried out in two stages, which showed a significant difference between fresh and settled sludge. Possible conditions for the use of sludge in the substrate are presented.

**Key words:** sewage sludge, substrate, LMWWTPs, bioindication, spent active sludge

## 1. Introduction

In the process of production and consumption, a significant amount of man-made waste is generated. Such made waste is generated. Such wastes include: waste from biogas production (waste biomass), calciumcontaining sludge from thermal power plants, wastewater sludge and others. These wastes have a significant impact on the environment, since they occupy large areas of arable land necessary for their disposal or storage, and thereby pollute them. Accumulation of a large number of sewage sludge is the most essential problem for Ukraine. Today, in Ukraine, the amount of accumulated sludge exceeds 5 billion tons, which increases by about 40 million tons annually [1]. Their dumps cover an area of more than 33 thousand hectares, and only from 3 to 5 % of these wastes are used as a secondary raw material, and the rest continues to accumulate [2].

Nowadays, there are many methods of sewage sludge disposal: incineration, dumping, use in various industries, etc. Methods for aerobic and anaerobic digestion are also used to dispose sewage sludge. In the case of aerobic conversion of sewage sludge, humic acids are formed in a greater amount, and in anaerobic conversion the main components are proteins and aromatic amino acids, indicating the possibility of using sewage sludge as organo-mineral fertilizers in agriculture. In most countries of the world, most of sewage sludge is used as the secondary raw material, for example, in the production of organo-mineral fertilizers. The share of sewage sludge used in agriculture is most significant compared to other methods of utilization. This is due to the fact that the sludge of wastewater contains a significant number of nutrients and growth substances such as phosphorus, nitrogen, potassium and others, and therefore it is a valuable organo-mineral fertilizer. One of the main restrictions on the use of sludge as waste fertilizers is the presence of a number of pathogenic microorganisms and eggs of helminths that are hazardous to animals and humans and many toxic substances, in particular heavy metals [4].

In world practice, burial is still the main way of sewage sludge disposal. However, in more developed countries, such as Portugal, Ireland, the United Kingdom and Spain, 70 % of all sewage sludge is used as a fertilizer in agriculture. In Sweden and Finland, sewage sludge is used for melioration and reclamation of the land damaged by industrial development [5,6]. In the United States, research is being carried out on the possibility of using sewage sludge as biofuel, as well as its transformation into the biological oil that can be fractionated into various types of liquid fuel including diesel and gasoline [7]. In Japan and the Netherlands,

much effort has been devoted to the extraction of phosphorus sewage sludge by applying a crystallization method. In addition, the newest technologies for the extraction of phosphorus have been developed, which are based on physico-chemical and thermal treatment of sewage sludge for dissolution of phosphorus, and then its restoration by burning [8, 9].

Many years of research have shown that sewage sludge can be used not only in agriculture as organic and mineral fertilizers, but also as an additive to building materials. Thus, according to [10], brick was made, which included a mixture of clay and shale with sewage sludge. The use of ash of heat treated sewage sludge in combination with clay is an excellent material for the production of light aggregates. In addition, the use of sewage sludge in road pavement and cement production is promising. The development of the possibility of using sewage sludge for the production of ceramic and glass products is also relevant. The results of the research show that in the products containing 5 % of sewage sludge their mechanical and water absorbing properties increase [11].

However, all these sludge disposal methods have one significant drawback – the operating costs for utilization of 1 m3. For example, for thermal drying, this figure is 20 euros, and for burning - 45 euros. These methods can also be applied to fresh sludge. And what is to be done with billions of accumulated sludge on the sites in Ukraine [12]?

Therefore, it is extremely important to find new ways of spent sludge disposal. We tested the hypothesis of the possibility of using a mixture of man-made waste containing an organic component to create a substrate that can be used in the biological reclamation of technogenically disturbed soils.

In Ukraine the need for biological reclamation at waste man-made objects (landfills, waste hedges, etc.) is increasing and this process requires considerable resource and financial costs. That is why the search for the means of its cheapening and preservation of natural resources is extremely promising.

In this way, we will be able to simultaneously influence the two extremely common environmental problems in Ukraine and offer an interesting technological solution for the use of nutrients from manmade wastes and provision of the process of biological reclamation.

The first step in the realization of our hypothesis is to check the quality of sewage sludge, to determine its composition and the possibility of using it in the substrate. The second, for the beginning, is the creation of a primitive substrate from a mixture of sewage and conventional sludge and the verification by means of bioindication of the possibility of growth and development of plants on this substrate.

Determination of soil quality is an integral part of modern agriculture. It is especially important to evaluate the quality of the soil after the reclamation of mine exits and industrial sites or when covering filled land excavations. Therefore, the definition of the state of the environment should be carried out not only with the help of chemical and physical methods, the use of which usually allows to determine only the content of individual pollutants, and thus does not always allow qualitative assessment of the overall impact of pollutants on living organisms. Biological methods should be used as well, namely bioindication, the advantage of which is that it enables to determine the joint biological activity of the influence of physical and chemical factors on the natural environment. In this method, preference is given to plants, because they are a very convenient object for biological monitoring of the state of the environment, since they characterize the state of the environment in which they grow, multiply rapidly, react differently to the action of harmful factors and thereby enable them to choose the most suitable appropriate response for a particular study [13].

## 2. Methods and materials

For the determination of qualitative parameters of sewage sediments, fresh sediments were selected, and To determine the qualitative parameters of sewage sludge, fresh sludge was selected, and studies were carried out according to generally accepted methods (Table 1) on certified equipment in the laboratory of agrochemical, toxicological and radiological studies of ecological safety of soils and quality of products of Lviv branch of the State Agency of Agricultural Research.

The research was carried out on wastewater sludge from Lviv municipal wastewater treatment plants. Two types of wastewater sludge were used: fresh and settled (stored in a sealed environment for 6 months, to simulate the conditions for the accumulation of sludge on the sludge sites).

Bioindication was carried out based on application and adaptation of the State Standards [14,15]. This method is suitable for all soils, soil-forming materials, deposited waste or chemicals that can be introduced into the soil. According to this technique, the growth substrates are the soil to be studied and the control soil, which is known to be of good quality. Two kinds of plants belonging to one category were chosen for the experiment. Category 1 – monocotyledonous plants: rye, rice, oats, wheat, barley, common sorghum, corn.

Category 2 – dicotyledones: white mustard, rape, radish and wild rape, Chinese cabbage, cress salad, tomato and beans. Before using the seeds of each culture, an analysis was made and the energy of their germination was determined. Ten identical seeds of the selected species were planted into each vessel. For each replication in each variant, the percentage of seed germination relative to average germination in control vessels was calculated. The length of the longest roots of each plant was measured and the average length of the longest root for each of the studied growth substrates was determined. A statistical analysis was used to determine the smallest significant differences between control and experimental concentrations.

The research was conducted in two stages:

**Stage 1**: Two experiments were carried out on settled sludge (fresh sludge sample was stored without oxygen for 6 months to simulate the conditions for waste dumping on sludge sites), to which normal dark gray soil was added.

In the first experiment, the settled sludge and soil were mixed in proportions of (%): 100:0; 80:20; 60:40; 40:60; 20:80; 0:100. On the created substrate, bioindication was performed by planting 10 seeds of spring barley (Hordeum vulgare), white mustard (Sinapis alba) and cress salad (Lépidium sativum) in Petri dishes. The experiments were carried out in a fourfold repetition.

In the second experiment, settled sludge, thermally treated settled sludge (2 h at  $t=105\,^{\circ}\text{C}$ ) and soil were mixed in proportions of (%): 60:40; 20:80; 0:100. Bioindication was carried out in Petri dishes by planting 10 seeds of spring barley into a substrate with heat treated sludge, and 10 seeds of spring barley etched with Vitavaks 200 FF, v.t.s. (normal consumption of 3 l/ton of seed). The experiment was carried out in a triple repetition.

**Stage 2:** The study was conducted on fresh sludge to which the usual dark gray soil was added in proportions of (%): 100: 0; 80:20; 60:40; 40:60; 20:80; 0: 100. Biodegradation was carried out in Petri dishes, planting 10 seeds of spring barley, etched barley (Vitawax 200 FF), not etched barley and cress salad. The experiment was carried out in a fourfold repetition.

During the experiment, observations were made on the following parameters: time of emergence of sprouts, their number for every day, total germination. Upon the completion of the studies the length and mass of the above-ground part and the roots were measured.

Depending on the results of the experiment, the possibility of using sewage sludge as a component of the growth substrate will be determined.

#### 3. Results and discussion

The primary task in our studies was to determine the quality of wastewater performance, the results of which are presented in Table 1.

The presented data show that there is a significant amount of the main nutrient elements (N -3.56, P -1.6, K -0.3%), macro- and trace elements in the sediment of sewage, as well as the available content of the organic constituent (23.8%), which can provide nutrients to most plants. The content of heavy metals in the samples studied did not exceed the MPC. The neutral reaction of the acidity of the medium should not have an inhibitory effect on plant growth and development.

Based on the results of the research we can make a conclusion about the conditionally safe chemical composition of sewage sludge for the use as a substrate.

To conduct the research on the possibility of using sewage sludge as a substrate with bioindication, we proceeded with the first stage of our research using settled sludge (the sample of fresh sludge was stored without access to oxygen for 6 months to simulate the conditions for storing waste on sludge sites).

After conducting two experiments of **stage 1** of bioindication studies, we obtained the following results:

- in the first experiment, where settled sediments were used, in all variants, in addition to control, no stairs were observed on all experimental crops;
- in the second experiment, where thermally treated and conventional settled sludge was used, the same pattern was observed: the use of etched seeds also did not produce results, experimental plant sprouts were not marked, except for the control one.

As a result of the **first stage** of the research, it was found that even a small fraction of sewage sludge (20 %) in the substrate, with this type of storage, has a very negative effect on the germination of plants used for bioindication. Fig.1 shows a typical sample, on which we can see the significant development of fungi and pathogenic microflora, which adversely affected the germination of seeds. Also in the second experiment, when using the heat treatment of sludge (2 h, at t == 05 °C), we observed that this thermal treatment is not appropriate, because it does not disinfect the substrate from the influence of fungi and pathogenic microflora, only reduces their number at the initial stage, and stimulates the activation of helminth eggs and the appearance of a significant number of worms (Fig. 2).

Table 1

Results of research of qualitative parameters of sewage sludge \*

T. P	Units of	Actu	al value	Normative document on test methods		
Indicator names	measurement	Dry substance	Natural humidity			
Acidity: pH salt	pН	_	6,4			
pH water	pН	_	6,1			
Wet	%	=	73,6			
Ash	%	23,8	_	DSTU 26712-85-		
Phosphorous is common	%	1,6	0,42	DSTU 26718-85		
Potassium is common	%	0,3	0,08			
Nitrogen is common	%	3,56	0,93			
Ammonium nitrogen	%	0,28	0,073			
Nitrogen nitrate (in peat)	mg/100g	11,75	-	DSTU 27894-88		
Calcium (as soil)	mmol/100g	11,75	_	DSTU 26487-85		
Magnesium (as soil)	mmol/100g	4,12	_	DS10 20487-83		
Sulfur is mobile (in the soil)	mg/kg	14,8	-	DSTU -26490-85		
Minerals:						
copper (Cu)	mg/kg	_	4,0			
zinc (Zn)	mg/kg	_	17,6			
manganese (Mn)	mg/kg	=	45,1	Methodological guidelines for		
cobalt (Co)	mg/kg	=	2,86	atomic-absorption determination		
iron (Fe)	mg/kg	=	65,0			
lead (Pb)	mg/kg	_	1,56			
cadmium (Cd)	mg/kg	_	0,20			
boron (B)	mg/kg	_	4,01	DSTU 10.154-83		

<sup>\*</sup> The research was conducted in the Lviv branch of the State institution "Soils protection institute of Ukraine"

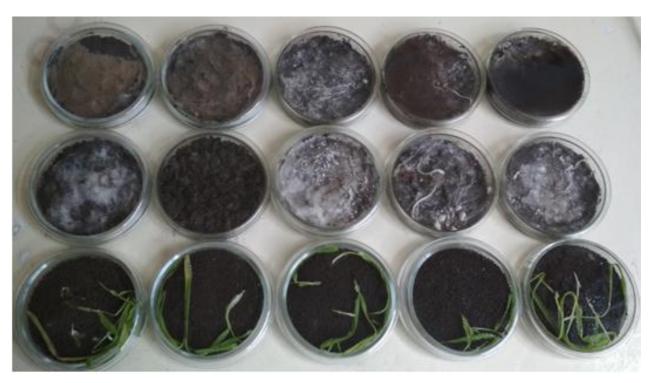


Fig. 1. The presence of fungi and pathogenic microflora in the studied samples

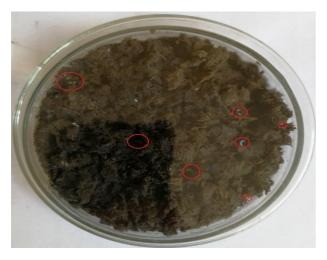


Fig.2. Option with heat treated precipitate

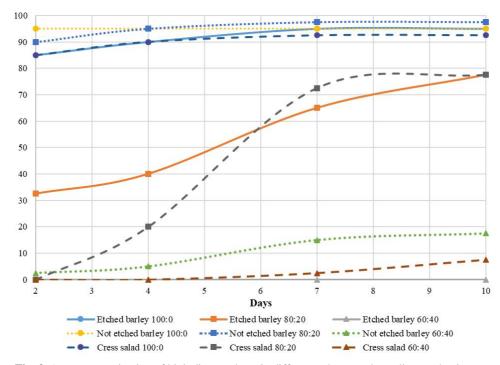
Consequently, the main problem of settled sludge is its decontamination since under such method of storage a significant amount of fungi and pathogenic microflora develops.

Summing up the results of stage 1, we proceeded to the **2nd stage** of the research using fresh spent sewage sludge.

The results of conducting bioindication on experimental mixtures of sludge with soil are presented in Table 2 and in Fig. 3. The studies have shown that germination of plants did not occur on the experimental substrates where the proportion of sludge exceeded 40 %, therefore these results were not reflected to make the presentation easier.

 ${\it Table~2}$  The data of germination of bioindicators in the investigated substrates

Date of the Option (soil:SS)		Similarity of experimental plants, %											
account	Option (soil:SS)	Etcl	hed Hor	deum vi	ulgare	Н	ordeum	vulgare	2	I	epidiu	m sativ	'um
26.09 (2 <sup>nd</sup> day)	Control 100:0	70	90	90	90	100	100	100	80	100	90	60	90
	Substrate 80:20	30	20	20	60	90	80	90	100	-	-	-	-
	Substrate 60:40	-	-	-	-	10	-	-	-	-	-	-	-
28.09 (4 <sup>th</sup> day)	Control 100:0	90	80	100	90	100	100	100	80	100	100	60	100
	Substrate 80:20	40	20	40	60	100	90	90	100	-	10	10	60
	Substrate 60:40	-	-	-	-	20	-	-	-	-	-	-	-
01.10 (7 <sup>th</sup> day)	Control 100:0	90	90	100	100	100	100	100	80	100	100	70	100
	Substrate 80:20	60	50	70	80	100	90	100	100	60	70	70	90
	Substrate 60:40	-	-	-	-	20	20	10	10	-	-	10	-
03.10 (10 <sup>th</sup> day)	Control 100:0	90	90	100	100	100	100	100	80	100	100	70	100
	Substrate 80:20	60	80	80	90	100	90	100	100	70	70	80	90
	Substrate 60:40	_	-	-	-	20	20	20	10	-	10	20	_



 $\textbf{Fig. 3.} \ \, \text{Average germination of bioindicator plants in different substrates depending on the time,} \ \, \%$ 

The results of the studies show that the conditionally acceptable amount of sewage sludge for the production of substrate under such conditions can be  $\approx 20$  %, since in all the bioindicative crops, the number of sprouts at the end of the experiment was not very different from that of the control one (etched barley – 17.5 %, cress salad – 15 %), but in not etched barley exceeded it (by 2.5 %). However, it should be noted that at the initial stages of the research (this is especially noticeable on cress salad and etched barley), the observed substrates showed a delay in the appearance of plants during the first 7 days of the study.

Adding 40 % of sewage sludge to the substrate negatively affected all the investigated variants. In etched barley, sprouts were not marked at all, in not etched barley and cress salad their amount was 17.5 and 7.5 % respectively. Therefore, in such substrate composition, it is not recommended to use such amount of sludge.

After receiving the sprouts, the determination of the influence of substrates on the development of plants was carried out, the data of which are presented in Table 3.

 $Table\ 3$  Changes in the growth and development of plants, depending on the use of different types of substrates

Version	Average height of the ground part of the plant, cm	Average root length, cm	Average weight, g	Average mass of ground part of the plant, g	Average weight of roots, g			
Etched barley (Hordeum vulgare)								
Control	10,83	18,19	0,239	0,155	0,084			
Substrate 1 (80:20)	8,51	11,12	0,252	0,147	0,105			
Substrate 2 (60:40)	=	=	=	=	-			
Not etched barley (Hordeum vulgare)								
Control	11,76	19,02	0,286	0,168	0,119			
Substrate 1 (80:20)	12,82	15,24	0,260	0,165	0,095			
Substrate 2 (60:40)	8,73	6,64	0,233	0,185	0,048			
Cress salad (Lepidium sativum)								
Control	4,36	5,31	0,0225	-	_			
Substrate 1 (80:20)	3,03	2,28	0,0205	=	_			
Substrate 2 (60:40)	1,98	0,65	_	-	-			

The obtained results of the dependence of changes in the growth and development of plants on the used substrates show that when using 20 % of sludge in the substrate, the total weight of plants does not vary significantly (from +5.4 to -9.1 %.) But at the same time, the difference in length is more significant. For the ground part it is from +9.0 to -30.5 %, and for the roots from -19.9 to -57.1 %.

When using 40 % of sludge in the substrate, there was a significantly greater effect on the growth and development of the plants, namely: the total weight of plants  $\approx$ 18.5 %, the height of the terrestrial part from -25.8 to -54.6 %, and the roots - from - 65.1 to -87.8 %.

#### Conclusion

The obtained results of the conducted studies indicate that sewage sludge contains a significant proportion of nutrients and can be used in the mixture for the creation of substrate. There is a problem with the use of settled substrates (which were stored for 6 months without oxygen), since even 20 % of the total amount has a detrimental effect on the bioindicative flora.

The use of 20 % of fresh sludge for the creation of a substrate does not cause much negative effect, and in

some cases, it has a positive effect on germination, growth and development of bioindicative plants.

Consequently, sewage sludge can theoretically be used to create a substrate in the process of biological reclamation of disturbed land, but it is necessary to find the appropriate amount of sewage. The results showed that under these conditions it should be  $\approx 20 \,\%$ . However, in our opinion, if the substrate is provided with other components of the system, for example, sorbents, it will be possible to increase the proportion of sludge in the substrate. We plan to carry out these studies in the future.

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