ORIGINAL PAPER

ASSESSMENT OF HEAVY METALS ACCUMULATION IN WHEAT GROWN ON SEWAGE SLUDGE-TREATED FERTILE SOIL

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Abstract. *The application of sewage sludge to agricultural land is an increasingly popular disposal route and it can be a valuable source of nutrients for crops. Soil properties such as texture, pH value, and ion exchange capacity, strongly influence the availability of trace metals to plants. This paper aims to determine the concentrations of heavy metals (Cd, Cr, Cu, Mn, Pb, and Zn) from wheat, grown in controlled conditions, using two analytical methods as Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analyzes were carried out at various stages of wheat grain growth and at different concentrations of sewage sludge/soil (0/100, 25/75, 50/50, 75/25, 100/0). The purpose of this paper is to present a method for valorizing sludge from sewage treatment plants, as well as the benefits that this method can have for the growth of cereals, while also looking at the influence and possible accumulation of heavy metals in this plant.*

Keywords: *sewage sludge; AAS; ICP-MS; crops; wheat.*

1. INTRODUCTION

Heavy metal pollution of the environment has been and is a permanent concern of scientists [1] as well as environmental protection agencies [2-3]. It is known that heavy metals can be found in a soluble form in water or are complexed with organic or inorganic ligands, a fact that radically influences their toxicity [4]. Existing sources of pollution can be divided into four main categories [5-7]: *i)* stationary sources (derived from domestic and industrial processes); *ii)* mobile sources (due to car traffic); *iii)* natural sources (due to volcanic eruptions, forest fires, etc.); *iv)* accidental sources (from accidental spills of products, industrial fires, etc.).

Knowing the characteristics and chemical composition of sludge resulted from urban wastewater treatment plants is very important in making decisions on their valorization [1, 8-9] or disposal [10]. The European legislation transposed at the national level also establishes strict criteria for the use of sludge in agriculture, at the same time stimulating the application of recovery methods [11]. Sludge recovery is random and disposal is often a significant operating expense. Traditional solutions, such as landfills and their incineration, have two main disadvantages [12]: a) the waste deposit can represent a risk of groundwater contamination and b) waste burning is a source of air pollution.

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The use of sewage sludge in agriculture, as well as increasing the quality of forest soils, are considered acceptable methods of use in the medium and long term in the European Union [11].

Indeed, the presence of toxic metals in sewage sludge can limit its application due to the risk of soil contamination and the transfer of toxic substances in the food chain [1, 13-16]. According to the Romanian National Water Administration (ANAR), in Romania, there are over 2,174 domestic, industrial and other types of wastewater treatment plants operating in a centralized system [11]. In addition, more than 1,789 individual small-scale treatment plants are registered, which currently produce approximately 210.450 tons of solid waste/year, an amount that is expected to increase by 2025 to more than 520,850 tons of dry matter/year. This forecast corresponds to the planned situation regarding the compliance of agglomerations in 2004, according to the National Implementation Plan of Directive 91/271/CEE on the treatment of urban wastewater [17].

The Sewage Sludge Directive (86/278/EEC) revised in 2000, mainly aims to establish the use of sewage sludge in agriculture in such a way as to prevent harmful effects on soil, vegetation, animals, human health, but also to support the correct use of it [18].

The elimination/utilization of sludge, as well as the reduction of pollution of environmental factors, is a priority for and for the Water Company Targoviste-Dambovita [19]. The Targoviste South Wastewater Treatment Plant within this company produces approximately 600-750 tons of dry matter annually. It is assumed that urban sludge, regardless of its type, can be used in agriculture, improving the organic matter in the soil and leading to plant growth [16, 20]. However, the diverse chemical composition of sludge can lead to different types of plant responses [21].

The purpose of this work is to present such a method of capitalizing these sludges, by using them in agriculture, taking into account the legislation in the field and the maximum loads of these sludges.

2. MATERIALS AND METHODS

2.1. MATERIALS

The universal soil type BIOFLOR.BC, hereinafter referred to as base soil, considered not to be contaminated by pollutants coming from cars with exhaust gas emissions or other sources of pollution, was used in this experiment. The sludge for the experiment was brought in January 2020, from the Targoviste South Wastewater Treatment Plant (Dambovita County, Romania).

2.2. METHODS

2.2.1. Physicochemical analyses

Before planting the wheat, an assessment of the quality of the soil and sewage sludge was carried out in terms of nutrient availability and their reaction (pH). The main characteristic of both media was evaluated according to generally accepted soil testing methods. The main characteristics of the soil are given in Table 1 and of sewage sludge is presented in Table 2.

Table 1. Basic characteristics of BIOFLOR.BC universal soil.

pH	5-7	Wood fiber weight ratio	0,.5-5%
Phosphorus	21 ppm	Humus content	3.1%
Potassium	190 ppm	Ash content	5%
Nitrogen	1.16%	Humidity	60-70%

Wheat samples were oven-dried (Binder FED 56) at 110℃ to constant mass and mineralized in an acid solution using a Berghoff MWS-2 microwave digestion system. The dried samples (200 mg) were placed in the digestion vessels; then 8 mL of nitric acid and 10 mL of hydrogen peroxide were added. After the digestion time (30 min), the vessels were cooled to room temperature and then each solution volume was brought up to 50 mL for each sample using deionized water.

For the mineralization of soil and sewage sludge samples, approximately 500 mg of dry sample was introduced into the digestion vessels and then 3 mL of nitric acid and 9 mL of hydrochloric acid (aqua regia) were added. After 60 min (digestion time), the dishes were cooled to room temperature and then each resulting solution was made up to the mark using deionized water (approximately 50 mL).

The concentration of heavy metals (Cu, Pb, Cd, Ni, Zn and Cr) was determined from the solution using the AA500 atomic absorption spectrometer (PG INSTRUMENTS LIMITED, U.K.) and iCAPQ quadrupole ICP-MS spectrometer (ThermoFisher Scientific, U.S). These two analysis methods were used to validate the obtained results.

2.2.2. Statistical analysis

For statistical analysis, it was used R language (version 4.0.3) in combination with R Tools 40 and Microsoft Excel softwares [1].

It was calculated the correlation between the concentration obtained by the ICP-MS with the concentration obtained by the AAS and to determined by the Pearson correlation index of the two programs. The correlations of Zn, Fe with various parts of the plant were calculated through Pearson correlation of both programs being obtained same results. It has been taken into account only the data that had (a two-tailed probability) < 0.01 or < 0.05 [27]. For each correlation $N = 4$.

The correlation coefficient is a statistical measure of the power of a linear relationship between the associated data [27-31].

Pearson correlation r type is widely used in statistics to measure the degree of association between variables and linear relationship [30, 31].

The Pearson r correlation of both variables should be normally distributed. Other assumptions include linearity and homoscedasticity. Linearity implies a straight relationship between each of the variables involved in the analysis, and in homoscedasticity is presumed that data are normally distributed in terms of the regression line [30].

The Pearson correlations between Cu, Pb, Cd, Ni, Zn, Cr on the samples analyzed by ICP-MS and AAS.

Principal components analysis provides additional information on the obtained data, but requires more complicated processing. This was done using the r language [31].

3. RESULTS AND DISCUSSION

3.1. RESULTS

The sludge was collected from a randomly selected drying bed where mixed sludges are subjected to dewatering. The principle is based on water filtration and evaporation from natural sludge in a drying area to minimize the water content of the sludge. This sludge is regarded as a stable, dry, solid state of particles with an average size of about 6 mm. The texture evaluation was performed as described by Robinson Khon [22].

Water content was expressed by weight as the ratio of the mass of wet to dry samples. The criterion for a dry sample was a constant weight of the sample after oven drying at a temperature between 100 and 110°C.

The organic carbon content was determined by the oxidation by dichromate, when it was treated with a few drops of diphenylamine dichromate remaining titrated with $FeSO_4$ solution, according to the procedure described by Anne [23].

Active lime was determined according to the Drouineau method [24]; the procedure involved reaction of the soil with ammonium oxalate, followed by determination of the unreacted oxalate by back titration with potassium permanganate.

The total amount of nitrogen was determined by the micro-Kjedhal method [25].

Phosphorus was determined by the spectrophotometric method with ammonium molybdate, according to SR EN ISO 6878/2005. The principle of the method consists in the reaction of orthophosphate ions with an acidic solution containing molybdate and antimony ions to form an antimony phosphomolybdate complex. Thus, the reduction of the complex is done with ascorbic acid and a blue-colored molybdenum complex is formed. The absorption measurement of this complex is done to establish the concentration of orthophosphate. Polyphosphates and some organophosphorous compounds are determined if they are transformed into orthophosphate activated with molybdate formed by sulfuric acid hydrolysis. Many organophosphorus compounds are transformed into orthophosphates by mineralization with peroxydisulfate. If a much more energetic treatment is needed, mineralization with nitric acid-sulfuric acid is used [1].

Potassium was determined by atomic absorption spectrometry by direct aspirating the filtered or digested and filtered sample into an air-acetylene flame [26].

Both averaged features were typical of their origin. The soil sample had sandy texture, alkaline pH and low organic matter content. The sewage sludge had a pH close to neutral and a significantly higher content of organic matter and major nutrients.

Table 2. The basic characteristics of the used sludge.

The attempt that has been made	Value/ U.M.	Test method
pH	7.94 unit. pH	SR EN 12176/2000
Phosphorus	10838 mg/kg s.u	SR EN ISO 6878/2005
Potassium	425 mg/kg s.u	STAS 12678/1988
Nitrogen	0.63%	ASTM D 5373/2008
Polycyclic aromatic hydrocarbons (PAHs)	2.92 mg/kg s.u.	SR ISO 13877-99
Total organic carbon (TOC)	17.78%	SR ISO 10694-98

Wheat was chosen as a test subject in accordance with OD No. 344/708/2004 [32] amended and supplemented by OM 27/2007 as well as due to its good growth characteristics and economic value. They were grown in a laboratory with controlled humidity, in labeled planters (Fig.1a) of 30x10x13 cm (LxWxH). The growth substrate for wheat was obtained from the mixture of a quantity of sludge (hereinafter referred to as addition) from the sewage treatment plant with BIOFLOR type soil (hereinafter referred to as base) as follows: 0% addition 100% base; 25% addition 75% base; 50% addition 50% base; 75% addition 25% base; 100% addition 0% base. Wheat seeds were planted in these planters. In order not to influence the results of the analyses, the plants were watered manually with double-distilled water.

a) b) c)

Figure 1. Wheat samples grown in controlled conditions.

After approximately 21 days from sowing (Fig. 1b), the first wheat samples (Fig. 1c) and substrate were collected. During the 2.5 months of the experiment, the effects of applying sludge in different concentrations for grain growth was evaluated by measuring the following biometric parameters: height increase, diameter increase at the base and average height diameter. The measurement of these parameters was carried out twice a month.

In general, biometric measurements increased with increasing proportion of sludge in the mixtures. The largest plants to be recorded were grown on a 50%/50% sludge mixture (Fig. 2).

Figure 2. Size of wheat after forty-five days grown period for the different concentrations of sludge in substrate.

Plants grown on a 50% silt mixture had a final average height of 35.4 ± 5.30 cm, and plants grown on the soil-base showed a lower average height of 20.3 ± 2.60 cm.

The differences were most obvious in the last 20 days, these results demonstrating good fertilization and improving the quality of the soil with sludge.

After 2.5 months of growth, the survival rate of the plants was approximately 80%, but being in laboratory conditions the plants did not reach full maturity (they did not have spikes).

The quantitative analysis of heavy metals (Cu, Pb, Cd, Ni, Zi, Cr) from the substrates used to grow plants as well as the plants grown on these substrates in different stages of growth was done by AAS and ICP-MS. For this, the substrate samples and the grains were dried in an oven and mineralized using the Berghof type microwave digester. The obtained results are given in Tables 3 and 4.

Table 3. Results obtained by AAS.

Sample	Description	Cu [mg/kg]	Pb [mg/kg]	Cd [mg/kg]	Ni [mg/kg]	Zn [mg/kg]	Cr [mg/kg]
Sample1	Base soil (B) 100% - Harvest 0 (blank sample)	13.809	10.674	11.25	59.427	38.935	13.025
Sample2	Addition of sludge (A) 100% - Harvest 0 (control sample)	268.379	76.657	15.875	214.606	455.701	149.663
Wheat							
Sample3	NGA_100 %	276.919	68.237	16.013	105.574	449.865	120.656
Sample4	NGB_2.25 %	77.787	22.964	10.251	62.555	196.001	44.834
Sample5	NGB_1.5 %	151.699	59.963	13.061	85.29	329.375	54.633
Sample6	NGB_ 0.75%	194.598	59.16	14.066	93.337	388.892	91.327
Sample7	NGB_100 %	15.424	26.419	11.556	49.094	33.228	12.895
Sample8	GT_B100	14.694	16.776	5.12	12.295	71.747	BD
Sample9	GT_B2,25	15.929	BD	8.325	22.213	82.724	72.857
Sample10	GT_B1,5	43.401	18.018	8.248	17.607	83.365	BD
Sample11	GT_B0,75	20.103	16.692	5.094	16.311	90.208	BD
Sample12	GT_A100	19.737	16.388	2.501	16.014	82.195	BD
Sample13	GR_B100	17.614	BD	4.91	27.511	60.047	BD
Sample14	GR_B2,25	27.875	18.185	6.937	26.656	139.994	BD
Sample15	GR_B1,5	54.735	BD	8.173	30.532	217.274	72.857
Sample16	GR_B0,75	51.755	BD	7.462	31.857	197.728	72.857
Sample17	GR_A100	98.259	36.63	8.384	49.218	316.169	BD
	RSD[%]	0.06-2.7	0.7-1.7	0.1-5.5	0.7-1.7	0.4-0.6	0.1-0.5
	CMA*	500	300	10	100	2000	500

BD-below the detection limit

**CMA-Maximum permissible concentrations of heavy metals in sludge destined for use in agriculture (mg/kg dry matter) as OD no. 344/ 16.08.2004*

**N= the substrate on which the grain grew; G = wheat; A=addition (sludge from sewage treatment plant); B=base(soil/earth); T-Stem; R-roots*

**0,75%; 1,5%; 2,25%;100%= mass percentages of B or A*

Table 4. Results obtained by ICP-MS.

Wheat							
Sample	Description	Cu [mg/kg]	Pb [mg/kg]	Cd [mg/kg]	Ni [mg/kg]	Zn [mg/kg]	Cr [mg/kg]
Sample3i	NGA_100%	278.817	69.102	16.811	106.342	461.562	121.816
Sample4i	NGB_2.25%	78.655	23.642	11.196	63.326	196.856	46.241
Sample5i	NGB_1.5 %	153.019	60.523	14.051	86.376	330.455	55.823
Sample6i	NGB_ 0.75%	196.108	60.116	15.246	94.673	340.312	92.427
Sample7i	NGB_100%	16.718	27.312	13.026	50.102	34.348	14.045
Sample8i	GT_B100	16.658	16.404	7.351	12.951	141.264	1.246
Sample9i	GT_B2,25	16.875	1.047	9.493	23.735	76.471	72.428
Sample10i	GT_B1,5	47.211	13.580	9.466	15.410	85.291	1.344
Sample11i	GT_B0,75	21.633	16.699	6.316	15.298	93.060	1.425
Sample12i	GT_A100	20.935	16.592	2.302	15.116	84.464	0.987
Sample13i	GR_B100	17.792	1.244	5.288	28.547	62.742	0.499
Sample14i	GR_B2,25	25.346	18.253	10.571	27.943	138.880	0.601
Sample15i	GR_B1,5	58.242	1.421	10.566	28.672	213.785	75.178
Sample16i	GR_B0,75	49.356	0.959	10.429	29.195	193.739	75.027
Sample17i	GR_A100	96.557	32.345	9.839	47.066	324.571	0.529
	RSD[%]	0.06-2.7	0.7-1.7	0.1-5.5	0.7-1.7	0.4-0.6	0.1-0.5
	CMA*	500	300	10	100	2000	500

**CMA-Maximum permissible concentrations of heavy metals in sludge destined for use in agriculture (mg/kg dry matter) as OD no. 344/ 16.08.2004*

***N= the substrate on which the grain grew; G = wheat; A = addition (sludge from sewage treatment plant); B = base(earth); T-Stem; R-roots*

****0,75%; 1,5%; 2,25%;100%= mass percentages of B or A*

The graphic representation of the results obtained on the wheat samples (substrate, root and stem) are given in the Figs 3, 4 and 5. This graphic representation was made to be able to highlight the concentrations of heavy metals existing in the substrate and in each part of the plant (root or stalk).

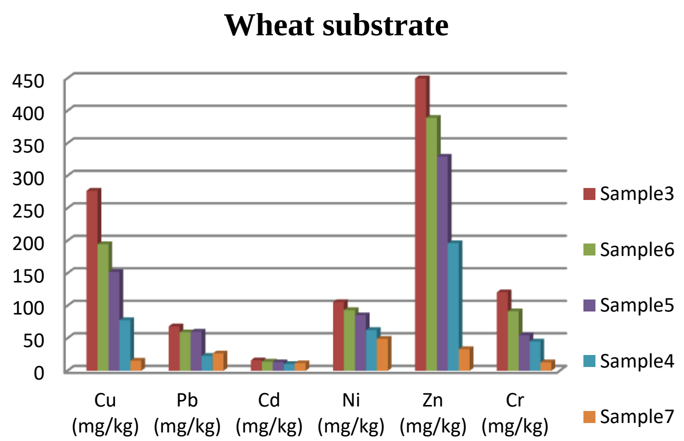

Figure 3. The heavy metal concentration from the wheat substrate

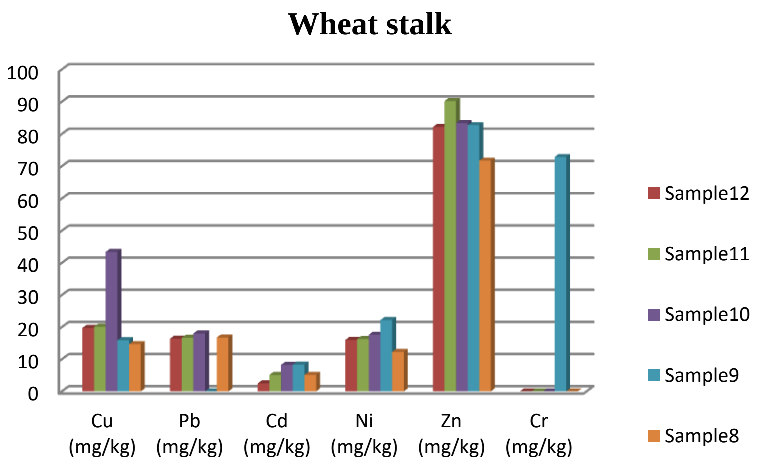

Figure 4. The heavy metal concentration from the wheat stalk

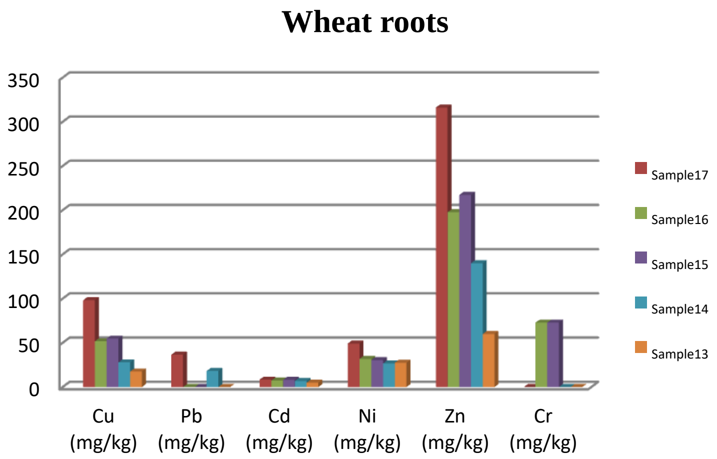

Figure 5. The heavy metal concentration from the wheat roots

3.2. DISCUSSION

The determinations related to the chemical composition showed that, in general, the wheat proved an exclusionary character. In other words, the level of heavy metals in the roots or stem was below their level in the soil.

Table 5. Pearson correlations of Cu, Pb, Cd, Ni, Zn, Cr concentrations in wheat substates (N = 4; p < 0,01).

	Cu	Pb	Cd	Ni	Zn	Cr
Cu	1	0.911	0.909	0.990	0.972	0.981
Pb	0.911	1	0.926	0.947	0.904	0.830
Cd	0.909	0.926	1	0.898	0.820	0.873
Ni	0.990	0.947	0.898	1	0.988	0.955
Zn	0.972	0.904	0.820	0.988	1	0.944
Cr	0.981	0.830	0.873	0.955	0.944	1

The level of heavy metals is also increasing in the wheat substrate (> 0.830, N = 5, p < 0,01).

Table 6. Pearson correlations of Cu, Pb, Cd, Ni, Zn, Cr concentrations in wheat roots (N = 4; p < 0,01).

	Cu	Pb	Cd	Ni	Zn	Cr
Cu	1	0.396	0.437	0.117	0.268	-0.325
Pb	0.396	1	-0.495	-0.809	-0.052	-0.997
Cd	0.437	-0.495	1	0.615	0.087	0.561
Ni	0.117	-0.809	0.615	1	0.498	0.833
Zn	0.268	-0.052	0.087	0.498	1	0.057
Cr	-0.325	-0.997	0.561	0.833	0.057	1

From Table 6 presented above, the following Pearson correlations can be observed:

- the increase in the concentration of lead in the wheat root is correlated with the decrease in the concentrations of nickel (-0.809, p < 0.01, N = 5) and Cr (-0.997, p < 0.01, N = 5);

- the increase in the nickel level is correlated with the increase in the chromium level (0.833, p < 0.01, N = 5).

Principal component analysis applied to Cu, Pb, Cd, Ni, Zn and Cr concentrations in wheat root is presented in Table 7.

Table 7. Principal component analysis applied to heavy metal concentrations determianted on wheat roots samples (N = 5).

	Component	
	1	2
Cu	-0.278	0.895
Pb	-0.989	0.143
Cd	0.593	0.563
Ni	0.871	0.421
Zn	0.146	0.621
Cr	0.995	-0.077

Through principal component analysis 2 principal components can be observed:

- component 1, characterized by the increase in the concentrations of cadmium, nickel and chromium in relation to the decrease in the concentration of lead;

- component 2, characterized by the correlation between copper, cadmium and zinc concentrations.

Such results can be explained by the presence of additional nutrients and organic matter in the sludge mixtures. Strong grain growth was induced by rapid nutrient consumption [33]. Michel and all. [34] indicates that the sludge could be used as fertilizer in agriculture or for forest soils.

On one hand, the application of sludge increased the concentration of trace elements in the substrate. However, certain trace elements in the substrate used here, for example lead, zinc and copper, can also be found in high concentrations in natural soil.

In the present study a positive effect of sludge modification for the wastewater treatment plant was found. Therefore, under similar climatic conditions, the effects of sludge application on agricultural or forest soils are expected to be even greater, due to the improvement of soil physical and chemical properties [35].

4. CONCLUSIONS

In general, sludge is considered to be a toxic substrate for the environment and agricultural production due to its high content of heavy metals, which limits its application as a fertilizer.

This study was carried out to find out the possible benefits of using the sludge from the Târgoviște Sud sewage treatment plant in agriculture, for growing wheat. The study is very important to assess the possible risks that may occur to domestic animals and human consumers.

To see the benefits of sewage sludge, wheat was grown in laboratory conditions without possible pollution from air, rain or soil influencing the results. An assessment of the quality of soil and sewage sludge used for growing wheat was included in this paper.

The results obtained on AAS and ICP-MS indicated a good correlation. However, in the sludge from the Târgoviște Sud wastewater treatment plant, heavy metals were present in small concentrations, which do not exceed the levels allowed according to the standards and regulations in force.

The results of our experiment demonstrated better fertilization properties when using a soil/sludge mixture with a silt content higher than 50%. Wheat had 20% better growth, showing that sludge can play an important role in plant growth.

At the same time, the statistical analyzes used indicated a good correlation regarding the concentration of Pb in the root which is directly proportional to the concentration of Ni and Cr in the plant. In the root Ni and Cr concentrations increased simultaneously.

The determinations related to the chemical composition showed that, in general, the wheat proved an exclusive character. In other words, the level of heavy metals in the roots or stem was below their level in the soil. This analysis shows that some of the heavy metals in the soil are consumed by wheat for its development.

So, sewage sludge from this sewage treatment plant can be successfully used for fertilizing forest land and urban landscapes. The application of sludge in agriculture and forestry appears to be a good option of use for this type of waste, together with its ability to improve the structure and fertility of agricultural and forestry soils.

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