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UTILIZATION OF SEWAGE SLUDGE FROM WWTP THROUGH PROCESSES OF COMPOSTING AND VERMICOMPOSTING

ABSTRACT

of dissertation for awarding the educational and scientific degree "DOCTOR"

Professional field 4.4. EARTH SCIENCES
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The Ph.D. thesis is written on 273 pages and contains 38 tables and 97 figures. The literature index includes 481 sources, of which 9 in Cyrillic and 472 in Latin. The paper was discussed and directed for the defence at the extended meeting of the Department of Microbiology and Environmental Biotechnology, Faculty of Plant Protection and Agroecology, Agricultural University - Plovdiv, held on September 28, 2021.

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The defence of the dissertation will take place on .... 2021 from .... h in hall № ……, Faculty of Plant Protection and Agroecology at the Agricultural University - Plovdiv. The materials related to the dissertation are available on the website of AU-Plovdiv.

I. INTRODUCTION

The process of wastewater treatment is associated with the formation of sewage sludge due to the biological stage of the urban WWTPs. The sewage sludge is a heterogeneous mass, which makeup varies significantly depending on the used technology, degree of purification, stabilization, etc. Nowadays, most of the designed and operating WWTPs in Bulgaria do not consider the problem called sewage sludge and theirs obligations regarding the wastes management arising Bulgarian and European legislation.

The sewage sludge is considered unnecessary and therefore must be subjected to landfill disposal. The non-hazardous sewage sludge formed in Bulgaria of 45 Water and Sanitation operators and 13 local WWTPs in 2018 is at an amount of 53082,62 t/DM, of which 4 810 t are generated in Plovdiv district (National Waste Management Plan 2021-2028). At the state level, 56% of the sludge is used in agriculture, 11% for reclamation of disturbed areas, 7% is landfilled, 20% is temporarily stored, and only 6% is subjected to composting processes.

The utilization of WWTPs sludge in agriculture may bring certain risks to the environment. Once applied, the metals are accumulated into the topsoil and can remain there for a considerable time or infiltrate deeply and cause contamination of surface and groundwater bodies. On the other hand, WWTP sludge is rich in organic matter, micro-, and macronutrients useful for agricultural production purposes. The high content of organic matter can improve the physicochemical and biological soil properties.

Taking the pathway of recovery and utilization, sewage sludge must be treated to make it a product suitable for use in agriculture. Such a recycling process, in which organic matter and other biodegradable components are used according to their original purposes, is composting. The process has been known since antiquity but has gained new significance in recent years.

An in-depth study of the possibilities for utilization of composted sludge in agriculture has not been carried out in Bulgaria yet. The potential for on-site sludge treatment is considerable due to the existence of a large number of small WWTPs, the process doesn't require significant investments, and the result is a product suitable for use in agriculture, landscaping, etc.

II. PURPOSE AND TASKS OF THE DISSERTATION

The problem analysis determined the **main goal** of the dissertation:

Improving the valorisation of sewage sludge from the urban WWTP through composting and vermicomposting and turning the potentially hazardous waste into safe products for the environment and beneficial for agriculture.

To achieve the main goal were set the following **specific tasks**:

1. Carrying out composting and vermicomposting of WWTP sludge along with other biodegradable waste from agriculture and urban landscaping activities.
2. Investigation of the obtained composts and vermicompost for presence or absence of phytotoxicity through seed germination tests.
3. Investigation of applicability of the obtained composts and vermicomposts in cultivating vegetables (tomatoes and peppers).
4. Compost and vermicompost quality compliance analysis to meet the requirements according to the *Ordinance on the separate collection of bio-waste and treatment of biodegradable wastes*.

III. MATERIALS AND METHODS

3.1. Experimental setting

This thesis has a practical focus, caused by the necessity for optimization and improvement at the recycling processes of an inevitable and persistent waste such as sewage sludge, formed by the biological stage during the wastewater treatment (code 19 08 05, Ordinance 2 for Classification). The fact is that the recycling (R3) of this waste is insufficient in our state.

Initially was performed composting of the WWTP sludge with various biodegradable wastes. The obtained composts were vermicomposted with earthworms. The final products obtained from both processes (composts and vermicomposts) were tested on vegetable crops for the presence or absence of phytotoxicity, according to the requirements of the *Ordinance on the separate collection of bio-waste and treatment of biodegradable wastes*. At the end of the study, the compliance analysis of results was performed with the legislation requirements (*fig.1*).

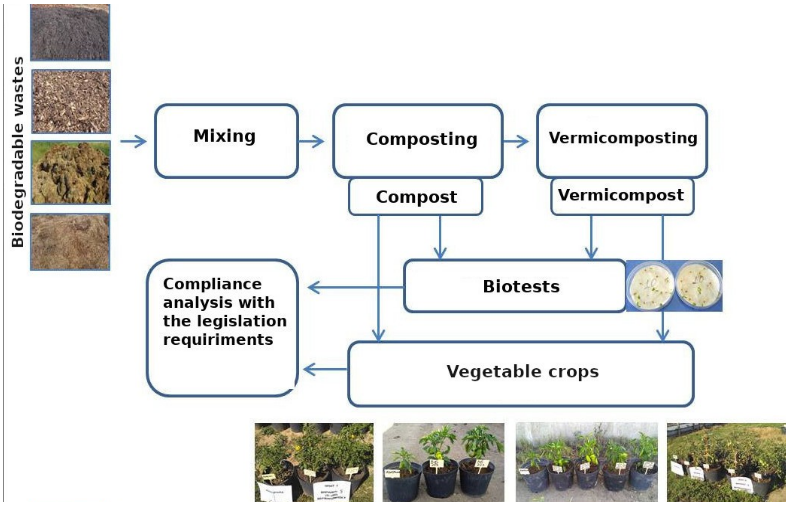

Figure 1 *The sequence of researches scheme*

3.2. Materials used in the experiments

Composting and vermicomposting were performed at the Biodegradable wastes recycling site, owned by company Bulplod Ltd., located at Kalekovets village, "Maritsa "Municipality, Plovdiv region. To obtain the composts were mixted at different proportions of sludge from WWTPs, cow manure, wheat straw, wood chips, grass clippings, and dry leaves. The sewage sludge used for the experiments came from two different batches of WWTP - Plovdiv. A local farmer delivered the cow manure and the wheat straw. The rest of the biodegradable wastes came from the landscape activities around the city of Plovdiv. The source materials were analyzed before the beginning of the experiments.

3.3. Technology of composting

The experiments were performed as per the windrow composting method (composting piles). The biodegradable wastes, in relevant ratios, were mixed and formed into compost piles (*fig.2*). The composting piles were 1,50-1,70 m x 2 m (height and width). The length was different for each experiment depending on the amount of available input materials.

The aeration of the composting piles was achieved by mechanical overturning with the front loader once per week. The moisture was measured daily and set up to 50-60%.

Three experiments were carried out in which the sewage sludge was mixed with various biodegradable wastes: (i) from agriculture; (ii) from urban landscaping activities; iii) a combined approach. The first experiment was set using biodegradable wastes from agriculture (manure and straw). Five shaped piles were made of different percentages of sewage sludge and cow manure (80:20, 60:40, 40:60, 100:0, 0:100), while the straw was used to refine the carbon concentration. The total amount of used material was: 14,2 t of sludge, 13,2 t of cow manure, and 9,2 t of wheat straw. The ratio between the different input materials is selected so that C:N = 27,21-27,75.

Figure 2 *Settling and mixing the composting piles*

For experiment 2, sewage sludge and other biodegradable wastes were used from urban landscaping operations (grass clippings and wood chips). The total amount of material used for the experiment was: 4,9 t of sludge, 3,6 t of wood chips, and 1 t of grass. The C/N ratios were adjusted to 26,32 at treatment 1 and 26,64 at treatment 2.

For the third experiment were used a combined approach was regarding the selection of biodegradable wastes. The investigation was conducted in two treatments: sludge (4 t), leaves (3 t), and chips (1,5 t), for those of treatment 2 - sludge (5 t), straw (1,7 t), and chips (2,7 t). The C/N ratio was established to be 26,40 for treatment 1 and 26,25 for treatment 2. Due to the relatively dry input materials, the water was added to provide adequate moisture for the biodegradation processes at the beginning of the composting. The duration of composting took 18-19 weeks.

3.4. Technology of vermicomposting

For the purpose of experiments, a mixed population of species *Eisenia fetida* (Sav.) and *Lumbricus rubellus* (Hoff.) were used. The species selection was determined according to a different tolerance to the medium's parameters and the heterogeneity of the used WWTP sludge. For the aim of vermicomposting, the total amount of 10 m² worms (1 m² contains between

30000 – 40000 worms) were used. Vermicomposting was set according to the "one batch reactors "technology. Rectors type "bed" and type "composter" were used (*fig.3*).

Figure 3 *Vermicomposting rectors*

The obtained composts of experiment 1 were subjected to vermicomposting on 30.10.2015 and experiment 2 on 15.12.2015. Both experiments used "bed-type" reactors. They were made of semi-permeable material to ensure adequate water drainage. Each of those "beds" had dimensions: 3,50 m length, 0,60 m width and 0,50 m depth. To each of them were added one m^2 (30000 – 40000) worms of a mixed population of *Lumbricus rubellus* (Hoff.) and *Eisenia fetida* (Sav.) species.

The obtained composts of experiment 3 were subjected to vermicomposting on 01.07.2016. For vermicomposting to ensure good aeration of the substrate and water drainage, one squire meter (100 x 100 cm) wood composters were used. To each composter were added 0,5 m^3 of compost from the respective batch (the layer in about 50 cm) and 0,5 m^2 mixed population of *E. fetida* (Sav.) and *L. rubellus* (Hoff.) species. The corresponding aeration regime for vermicomposting was maintained by mechanical turning, performed weekly using a pitchfork. The suitable moisture of 70-75% was achieved by irrigation and shading.

3.5. Study of the impact of composts and vermicomposts on experimental plants

The main goal of the conducted biotests was to evaluate the impact of the obtained composts and vermicomposts on the seed germination, growth, and development of the selected species. Laboratory tests for germination were performed with watercress seeds (*Lepidium sativum* L.) and green pea seeds (*Pisum sativum* L.), variety "*Meteor*".

The field biotests with vegetable crops of tomato seedlings (*Solanum lycopersicum* L.) variety "Milana" and pepper seedlings (*Capsicum annuum* L.) variety "Kurtovska Kapiya" were carried out. The field biotests were set at two concentrations, respectively 25% and 50% compost or vermicompost (v/v).

3.6. Mathematical and statistical data processing methods

All obtained results are processed using open-source software for Linux – LibreOffice calc and LibreOffice math, version 6.3.0.4. The diagrams were created by using GnuMetric version 1.12.35. All images used were processed via GIMP, version 2.10.12. A one-way analysis of variance (ANOVA) with SPSS software (SPSS Statistics, USA) was used for data processing.

IV. RESULTS AND DISCUSSION

4.1. Composting and vermicomposting of WWTP sludge and agricultural biodegradable wastes

At the present experiment, was observed the typical curve for composting processes. It was characterized by a rapid increase in temperature, reaching the thermophilic phase and increasing the amount of released CO_2 at the very beginning of the process (first week). After day 70, CO_2 values were established at relatively low levels, which corresponded with the decrease at temperature during this period (after the first 65-70), resulting from the depletion of easily biodegradable organic substances and slowing down the intensity of the process (*fig.4*).

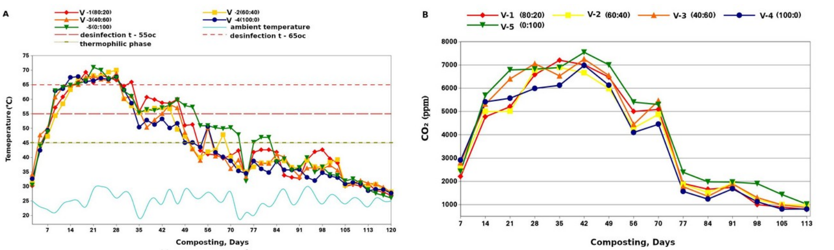

***Figure 4** Changes in temperature (A) and released CO2 in-situ (B) from the composting piles of experiment №1*

The intensity of the released CO_2, under laboratory conditions, showed high values during the composting process at all five treatments. At the end of the process (after 120 days of composting) CO_2 emissions decreased significantly. The lowest values were measured at the vermicomposts. The concentration of CO_2 released from the final compost had been incremented, increasing the concentration of manure in WWTP sludge. An inverse relationship was observed between the vermicompost and the compost.

There was found a higher reduction of CO_2 emissions in the treatments with a higher initial sludge:manure ratio (*fig.5A*). During vermicomposting, both earthworm species *Eisenia fetida* (Sav.) and *Lumbricus rubellus* (Hoff.) were beneficial for the compost transformation. Their involvement led to organic matter stabilization and reduction of C/N ratio, which are leading indicators of reduced biological activity and achievement of compost maturity. As a result of vermicomposting, there was practically a leveling of the released CO_2 at the end of the stage. In contrast, in the beginning, increasing the manure concentration, the CO_2 release was also increased.

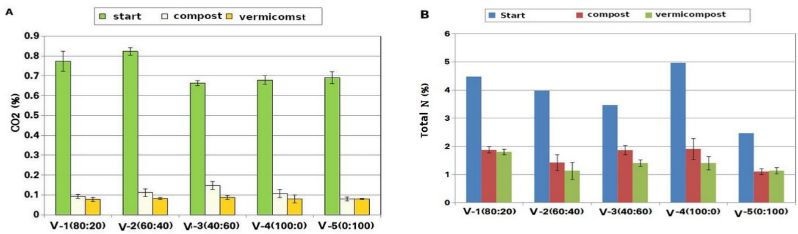

Figure 5 *Amount of released CO_2 measured by the "closed vessel" method (A) and amount of total nitrogen (B) at treatments of experiment №1*

After 120 days of composting, a significant decrease at a nitrogen concentration in all of the treatments was observed compared to the initial state. This trend continued at a slower pace during vermicomposting, as the established values were proportional to those obtained at compost (*fig.5B*). The nitrogen loss depended mainly on an added carbon material and C/N ratio. The nitrogen loss was higher at treatments with nitrogen-rich materials (WWTP sludge) and continued during composting and vermicomposting. At these treatments, the process was longer, while the stabilization was significantly slower.

The sludge-free treatment V-5 (0:100), which initially had the lowest percentage of nitrogen, achieved the fastest stabilization. The amount of total nitrogen found at compost and vermicompost of this treatment had similar values. At the same time, the concentration of organic matter also decreased, which is a natural result of biodegradation (*fig.6A*). The initial minor loss at the treatments containing WWTP sludge and a large amount of straw may be attributed to a higher percentage of stable organic components. During the vermicomposting, the reduction processes continued slowly.

The lowest concentration of organic matter and nitrogen in both composts and vermicomposts was found at treatment 5 (0:100). At this treatment, the highest temperatures and the highest amount of released CO_2 were observed as a result of the relatively higher microbiological activity and the absence of sludge. The final results of composts and vermicomposts from all five treatments meet the requirements for compost quality regarding the organic matter indicator (15-50%) according to the *Ordinance on separate collection of biowaste and treatment of biodegradable wastes*, 2017.

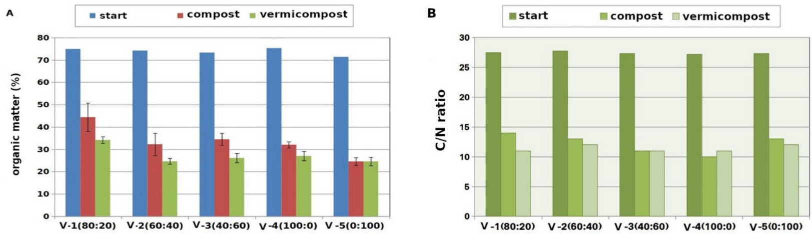

Figure 6 *Organic matter (A) and C/N ratio (B) at treatments of experiment №1*

The carbon loss was more significant than the nitrogen, so the C/N ratio decreased (*fig.6B*). At the final composts and vermicomposts, C/N values below 15 were observed at all five treatments, which is a sign of relative maturity. At treatments composed of sludge and manure, a decrease at the C/N ratio was observed with the manure content. At the end of composting the humification processes of treatments from 1 to 4 was in the advanced stage. So, the vermicomposting has no significant effect and the reduction of the C/N ratio is at the reverse order, from treatment 4 to 1.

The electrical conductivity of the treatments decreased over time (*fig.7A*). The final values for all five vermicomposts met the requirements for stability of compost in accordance with the *Ordinance* (≤3 $mS.cm^{-2}$). In addition, the pH of the used sludge was 6,98, i.e. neutral. After 120 days of composting, all five treatments had relatively different values (*fig.7B*). Still, the pH was lowest at the treatments with the highest sludge content (V-1 and V-4), which is a sign of an unfinished biodegradation process.

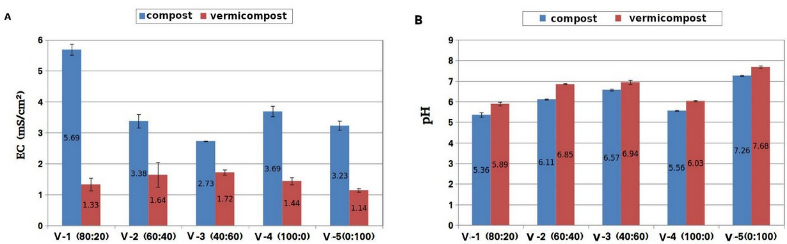

Figure 7 *Electrical conductivity (A) and pH (B) at treatments of experiment № 1*

During the application of the technology, several changes occurred related to the concentration of the studied heavy metals into the products of both processes (*fig.8*). A significant decrease in the concentrations of most heavy metals is observed in composts of some of the treatments. This was mainly due to bulk biodegradable materials (straw) for C/N ratio regulation at the beginning of the process. The volume of the compost piles is significantly increased, and the expected initial concentration of heavy metals was significantly reduced. The percentage of straw added to the total volume of the treatments was 26,5% at V-1 (80:20), 23,5% at V-2 (60:40), 18,5% at V-3 (40:60), 30% at V-4 (100:0) and 11,5% at V-5 (0:100). On the other hand, the increase in the concentration of some heavy metals (Ni, Cr, Pb, Zn, and Cu), in the compost of V-5 (0:100) can be attributed to the decrease at the initial volume. This was due to the intense microbial activity leading to significant mineralization of organic fractions and significant losses of C in the form of CO_2.

The observed results of heavy metals concentrations reduction after the vermicomposting can be explained by the ability of earthworms to accumulate certain metals in their tissues. In addition, the reduction in heavy metals concentrations can be attributed to the infiltrate leakage during vermicomposting due to precipitations and irrigations. At some treatments (V-2, V-3, V-5), the heavy metals concentrations in vermicomposts increased. Due to the active biodegradation, these are the treatments with relatively less or no sludge, less straw added, and the most considerable volume reduction in composts and vermicomposts. A certain amount of manure may stimulate microorganisms' development related to the biodegradation of organic matter.

The final results of metal content in composts and vermicomposts at all five treatments showed a decrease on the one hand due to the addition of large amounts of biodegradable materials (straw) with low metal concentrations and possible accumulation of used worms species. Vermicomposts from treatments V-2(60:40), V-3(40:60), V-4(100:0) V-5(0:100) met the requirements for product *compost* in terms of Cd, Cu, Ni, Pb, Cr, Zn content, according to the *Ordinance*. The lead content at V-1(80:20) exceeded the limit values about product *compost* of 180 $mg.kg^{-1}$ but met the product *organic soil improver* requirements of 250 $mg.kg^{-1}$.

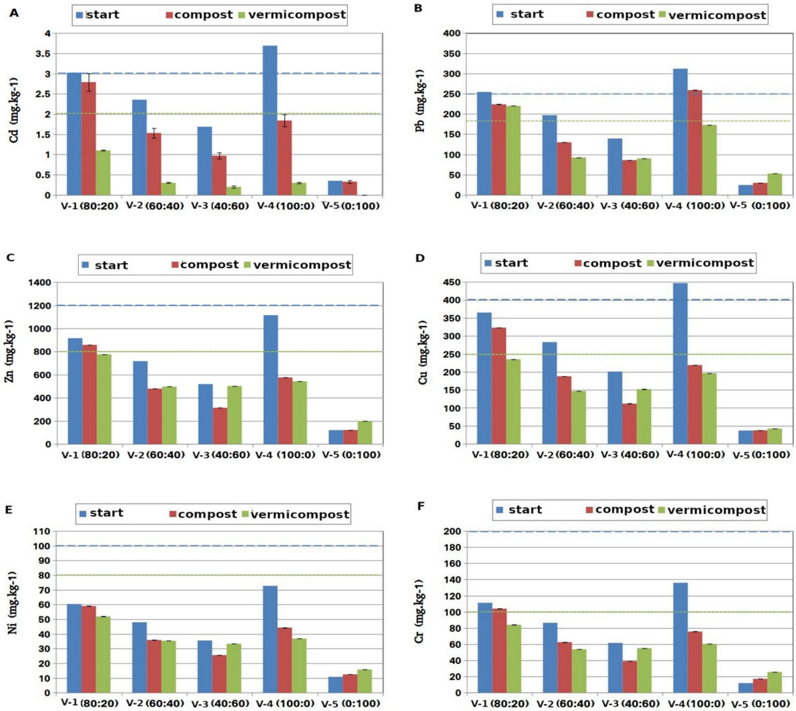

***Figure 8** Cd (A), Pb (B), Zn (C), Cu (D), Ni (E) and Cr (F) content at the imput materials, compost and vermicompost at the treatments of experiment №1*

The analyzes showed the absence of *Salmonella* sp. in a sample of 20 g, both at the used sewage sludge and at final composts and vermicomposts. On the other hand, *Escherichia coli* was present in 0,001 g of the used sludge. At composts and vermicomposts, the minimum volume of the pathogen was detected is 1 g at all five treatments. The third microbiological indicator was existence of *Clostridium perfringens*. The pathogen was detected in 0,001 g at the sludge and final composts and vermicomposts of all five experimental treatments. The constant concentration of *Clostridium perfringens* is probably due to the ability of this gram-positive bacterium to form endospores, which can survive for a long time under adverse conditions.

4.2. Composting and vermicomposting of WWTP sludge and biodegradable wastes from urban landscape operations

In the beginning, a rapid increase of temperature and the released CO_2 was observed, corresponding to the rise of microbial activity (*fig. 9A*). The thermophilic phase lasted between 75 and 80 days and was shorter in V-1. The reduced microbial activity can explain the relatively lower temperatures due to the higher sludge concentration (60% of the volume compared to 45% at V-2). As was observed at some of the treatments of experiment №1, the addition to the composting system of a large sludge amount, a material with the ability to absorb and retain water, is a premise for a higher moisture. The result was a shorter thermophilic phase and reduced microbial activity. The peak values of 67 °C (V-1) and 72 °C (V-2) were reached between day 18 and day 20. In order to regulate the temperature regime and avoid excessive temperatures during the next few weeks, mechanical overturnings (cooling) of both treatments were repeatedly carried out.

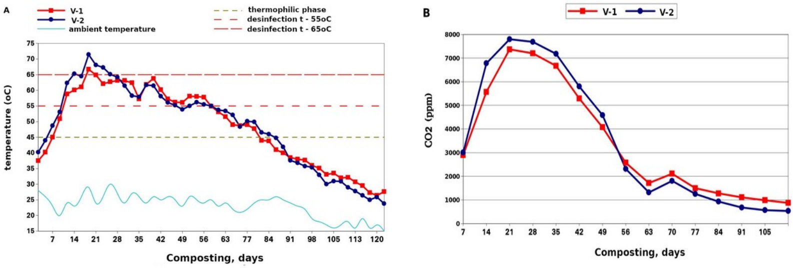

Figure 9 *Change in temperature (A) and dynamics of released CO2 in-situ (B) from composting piles of experiment №2*

The peak of CO_2 emissions coincided with the temperature (*fig.9B*). By day 54, higher levels of CO_2 emissions were found in V-2, which can be explained by the presence of a large amount of grass clippings, a relatively easy biodegradation material, compared to wood chips, which is the main source of carbon at V-1. After depletion of the readily biodegradable cellulosic materials, the amount of CO_2 gradually began to decrease, and during the second part of the process, the CO_2 released from V-1 was higher.

After 120 days of biological treatment at both treatments was observed a considerable decrease in the total nitrogen concentration, compared to the initial state (*fig.10A*). The nitrogen loss at V-1, containing a higher percentage of sludge, is greater and continues during composting and vermicomposting. At this treatment, the temperature and the amount of CO_2 emissions are relatively lower, and the stabilization of the matter is slower and takes longer. The data correspond with the results obtained at the treatments of experiment №1 that contained a higher amount of sludge.

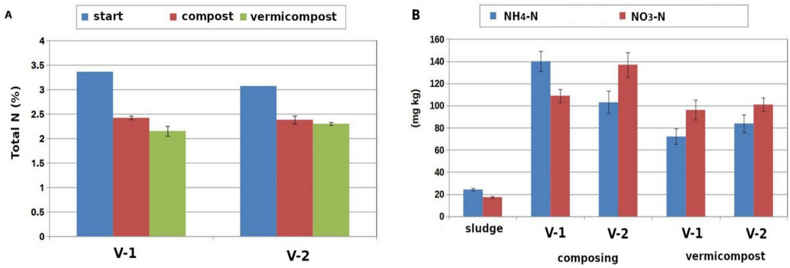

Figure 10 *Total nitrogen (A) and ammonium and nitrate nitrogen (B) content at the treatments of experiment №2*

As a result of the ongoing aerobic biodegradation and nitrification, ammonium and nitrate nitrogen content at both treatments increased significantly (*fig.10B*). At the V-1 final compost, NH_4-N was still predominant compared to NO_3-N, which shows a slow or incomplete nitrification process. At the other hand, NO_3-N was predominant in the final vermicomposts, and its content was lower than at composts, probably due to infiltrate leakage during the vermicomposting.

As at experiment №1, the results at experiment №2 showed a downward gradation of the organic matter content, which is a natural result of biodegradation (*fig.11A*). During composting, the values decreased, and at the end of the process, the organic matter content was higher at V-1 (56,8%) than V-2 (52,6%). During vermicomposting were found only a slight decrease at the indicator values. The presence of significant amounts of difficult-to-degrade materials required a longer biodegradation process to reach the necessary level of stability. Even at the end of the vermicomposting, undecomposed aggregates of wood chips were observed into the material of both treatments. According to the *Ordinance*, the vermicomposts results regarding the organic matter indicator almost met the compost quality requirements (15-50%).

An essential parameter for determining the stability and maturity of composts and vermicomposts is the C/N ratio. At V-1 there was a decrease in the indicator to 14:1 after 120 days of composting, and at V-2 the value was 13:1. Similar to the treatments of experiment №1, here, during composting, the loss of C was greater than that of N, so the C/N ratio of the compost piles decreased (*fig.11B*). After vermicomposting, the C/N ratio at V-1 remained the same - 14:1 and at V-2 was found to be 12:1. According to the obtained data, vermicomposting does not significantly affect the C/N ratio reduction, mainly when the composting system includes a large percentage of complex biodegradation materials.

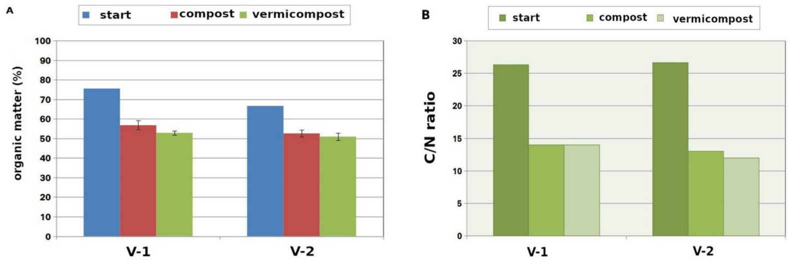

Figure 11 *Organic matter content (A) and C/N ratio (B)*

As a result of biological treatment, several changes occurred related to the concentration of the investigated heavy metals at the final products of the processes, composts, and vermicomposts (*fig.12*). At composts, a slight decrease in the concentration of some heavy metals (Pb, Cu, Ni, and Cr) was observed. This was mainly due to a large percentage of bulk biodegradable materials (wood chips and grass clippings) added to regulate the C/N ratio. In this way, the volume of the compost piles was significantly increased and the initial concentrations of heavy metals were reduced. As expected, the concentrations of heavy metals at V-1, containing more WWTP sludge, were higher. There was an increase in the concentrations of Cd and Zn, which can be attributed to a certain decrease of the initial volume (especially at V-2).

After the vermicomposting, increased Cd, Cu, Cr, Zn and Ni or values close to those measured at compost were observed at V-1. The only increase in lead concentration was found at V-2. These results corresponded to the data from the literature, where is mentioned the possibility of a significant increase of heavy metals concentrations during the biodegradation process. It was due to intense microbial activity, significant losses of C in the form of CO_2, and therefore the volume reduction of composting piles. The reduction of heavy metals at V-2 can be attributed to the possibility of infiltrate leakage during the vermicomposting process and to the accumulation of heavy metals into the earthworm tissues.

According to the *Ordinance on separate collection of biowaste and treatment of biodegradable wastes*, the products obtained after the two phases of treatment at V-1 did not meet requirements regarding the maximum permissible concentrations at two indicators. The values of Cu (250 $mg.kg^{-1}$) were lower than those found at compost from V-1 of 252 $mg.kg^{-1}$ and those at vermicompost of 256 $mg.kg^{-1}$.

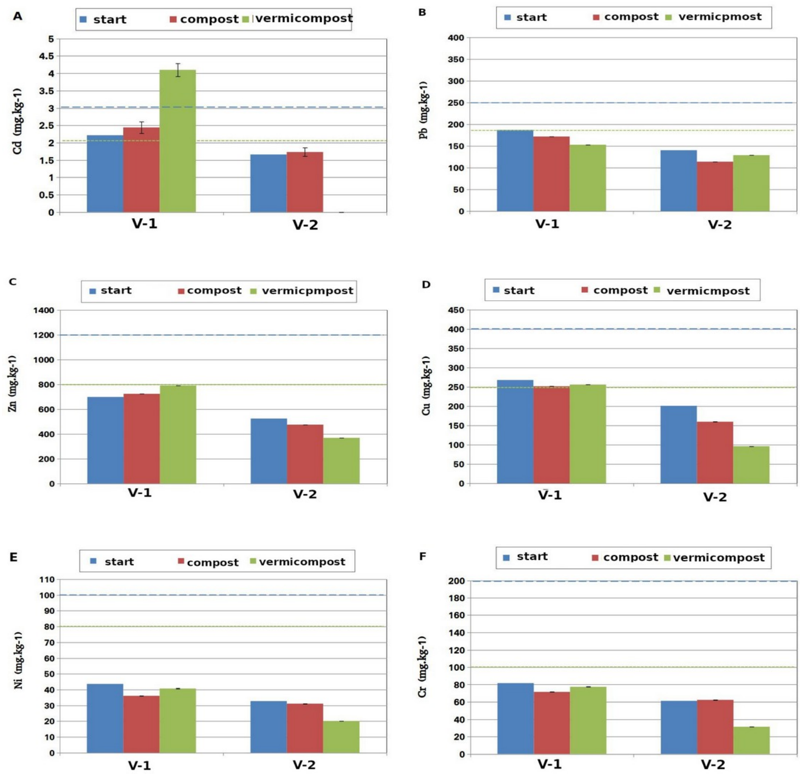

Figure 12 *Cd (A), Pb (B), Zn (C), Cu (D), Ni (E), and Cr (F) content into the input materials, compost, and vermicompost*

The limit concentrations values of Cd (2 mg.kg-1) were significantly lower than those found at V-1 compost (2,44 mg.kg-1) and V-1 vermicompost (4,1 mg.kg-1). The V-1 vermicompost also did not meet the requirements for an *organic soil improver*, where the limit values for Cd were 3 mg.kg-1. On the other hand, both the compost and the vermicompost of V-2 met the maximum permissible concentrations of the product *compost*.

It should also be noted that after 120 days of composting at V-1 the wood chips remained largely undegraded with a volume close to the initial and active microbial activity. V-2 also had undegraded particles of wood chips, but the grass clippings were completely decomposed, and the pile's temperature was lower than V-1. During the vermicomposting, the material of V-2 retained more moisture due to the higher percentage of well-degraded material, and the mixed worm population was significantly improved comparing to V-1.

Salmonella sp. was not found in the 20 g sample of used sludge, composts, and vermicomposts of both treatments. *Escherichia coli* was present in 0,001 g of used sludge, while at composts and vermicomposts, the minimum volume was 1 g. On the other hand, *Clostridium perfringens* was detected in 0,001 g of used sludge, and its concentration did not change at composts and vermicomposts of both treatments.

4.3. Composting and vermicomposting of WWTP sludge – a combined approach

At the end of the third week, a sharp temperature rise was observed, so all composting piles reached the values between 49-55 °C (*fig.13A*). In treatment 1, temperatures above 55 °C during 5-9 days were observed, but any of them did not reach temperatures above 65 °C. At treatment 2, high temperatures last relatively longer. Temperature above 55 °C lasted between 27 and 34 days, but temperatures above 65 °C for three days were noted only at V-2. The relatively lower temperatures compared with the first two experiments can be explained by the unfavorable environmental conditions during its conducting (the winter season).

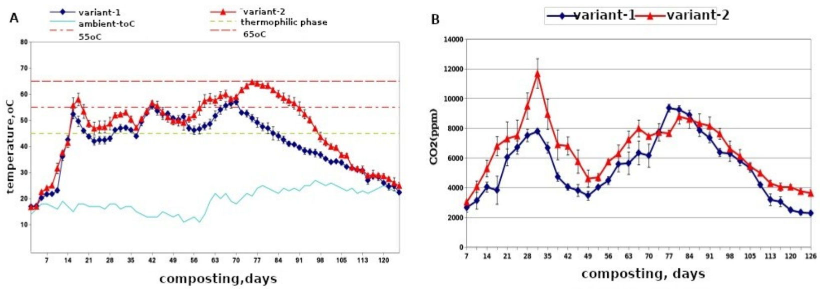

Figure 13 *Dynamics of temperature (A) and CO2 emissions (B) during composting*

At the beginning of composting, a rapid increase in the amount of released CO_2 was observed at both treatments, corresponding to a rise in microbial activity (*fig.13B*). The peak of released CO_2 was at the end of the third week, as treatment 2 is significantly higher than treatment 1. This corresponded with the rise in temperature during this period. The lowering of the temperature after stirring led to a slowing down of the processing speed and, respectively, lowering the release of CO_2. Subsequently, as a result of easily degradable organic compounds depletion, a permanent decrease in CO_2 emissions was observed. A positive correlation between the parameters (t, CO_2) was monitored *in-situ* during the composting process.

The amount of total nitrogen (*fig.14A*) was proportional to the amount of raw used materials, respectively, a higher amount of nitrogen was found at the treatment with more WWTP sludge (V-2). Unlike the previous two experiments, by day 67 there was a significant increase in the percentage of total N at both treatments. According to the scientific literature, such an increase could be explained by a lower intensity of biodegradation, reduction of composting piles volume or immobilization of N. Subsequent analyses showed the expected reduction at total nitrogen concentration. A more significant reduction was found at iterations of V-1. The measured values after vermicomposting were significantly lower than those found at the compost.

At the second measurement (day 67), a slight increase in the total phosphorus content was noted (*fig.14B*). Subsequently, by the end of the composting process, the total phosphorus content was decreasing at both treatments. At the end of vermicomposting, an increment was observed. According to the available literature, it could result from more intense mineralization of organic matter or the transformation of insoluble P into a more soluble form. Interestingly, the mobile forms of phosphorus at vermicomposts, compared to composts, increased by over 50%.

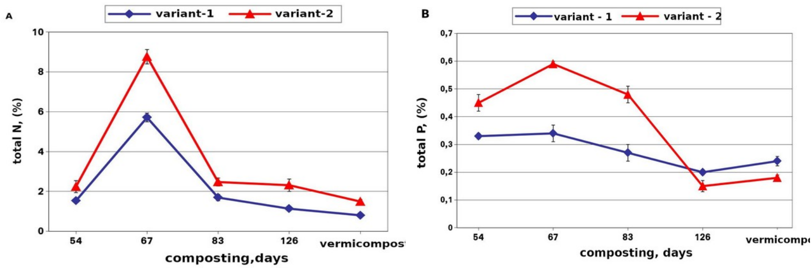

Figure 14 *Total nitrogen (A) and total phosphorus (B)*

The WWTP sludge had a concentration of 263 mg.kg^{-1} for NO_3-N and 127 mg.kg^{-1} for NH_4-N, respectively. After the first 54 days of composting, we observed an increase in NH_4-N concentrations (*fig.15A*), while the concentration of NO_3-N at both treatments was relatively low (*fig.15B*). During the second part of composting, the concentration of NH_4-N started to decrease, while the concentration of NO3-N increased due to the vigorous development of nitrifying bacteria.

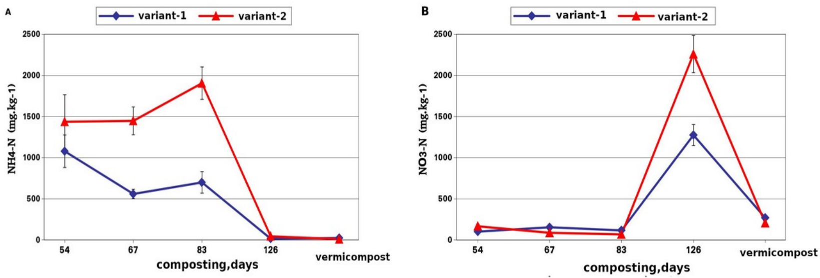

Figure 15 *Ammonium (A) and nitrate (B) content*

At the end of vermicomposting, the ammonium nitrogen continued decreasing due to an ongoing nitrification process. The decrease was proportional to this observed at the compost. The NH_4-N values were higher at the treatment with more sludge (treatment-2). The NO_3-N also showed a significant reduction during vermicomposting due to infiltrate leakage.

At the beginning of composting, a downward gradation of the indicator organic matter was observed, a natural result of biodegradation (*fig.16A*). The slight increase found at the analysis on the 67th day of the process can be attributed to the heterogeneity of the composting piles and the high content of difficult to decompose materials, such as wood chips. Therefore, treatment 2, which contains a higher percentage of wood chips, remained with a higher concentration of organic matter until the end of the process. On the other hand, dry leaves and straw were relatively easier to biodegrade and were completely degraded. The conversion of organic matter continued during vermicomposting. The observed intensive loss during vermicomposting can be attributed to earthworms (disintegration, homogenization, aeration of the composting mass).

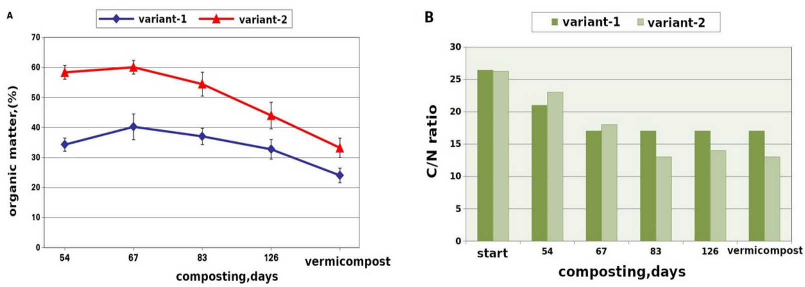

Figure 16 *Organic matter content (A) and C/N ratio (B)*

Another critical parameter is the C/N ratio, set at 26,40:1 for treatment -1 and 26,25:1 for V-2 at the beginning of the process (*fig.16B*). There was a decrease in the C/N ratio over time due to the intense loss of C during the biodegradation. The final compost C/N ratio of V-1 was established to 17:1, while of V-2 was 14:1. The followed vermicomposting did not lead to significant changes at this parameter.

The increase or decrease at EC during composting process indicates a increase or decrease at process intensity. The data obtained showed a decrease over time, which was more considerable at treatment-1 (*fig.17A*). The established final values at vermicomposts of 1,4 mS/cm^{-2} at V-1 and 1,65 mS.cm^{-2} at V-2 met the requirements for compost stability set in the *Ordinance*, 2017 (≤ 3 mS.cm^{-2}).

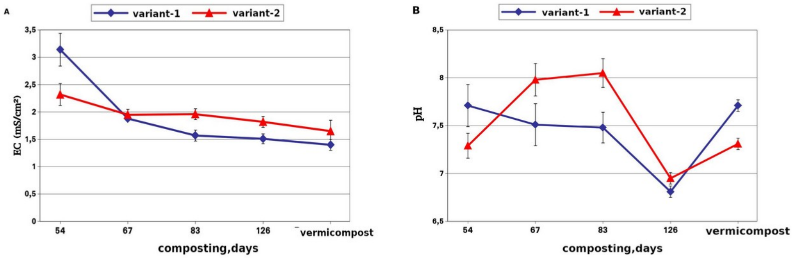

***Figure 17** Electrical conductivity (A) and pH (B) of experiment №3*

pH of WWTP sludge was neutral – 7,11 (*fig.17B*). At the end of the composting process, the measured values were lower, 6,81 and 6,95, respectively. The final values of this parameter at the vermicompost were 7,71 and 7,31, respectively. pH values can be related to the stabilization of vermicompost and its remissive microbiological activity.

The enzymatic activities ß-glucosidase and dehydrogenase were studied at dynamics. Due to the presence of carbohydrates, an increase in β-glucosidase activity (fig.18A) was observed during most of the composting time. After depletion of the main depots, the formation of enzymes by microorganisms was slowed down. As a result, the temperature of the piles decreased. The data correlated with those of the temperature and CO_2 emissions. At V-1, the temperature has been dropping since day 70th, from 56 ºC to below 45 ºC for two weeks, as the process leaves the thermophilic phase. A similar pattern of change around day 83 is observed at almost all indicators, total nitrogen, NH_4-N, NO_3-N, total P, pH, organic matter, microbial biomass, which showed the transition to the maturation phase and depletion of easily digestible organic compounds. During this period, the most important indicators describing the nutrient balance were higher at V-2 than a V-1, which indicates an incomplete first phase of biodegradation.

The dynamic of dehydrogenase activity (*fig.18B*) showed an earlier decrease at V-1 than V-2. This can be attributed to the higher cellulose content. The vermicomposting process also led to a reduction of enzyme activity and dehydrogenases.

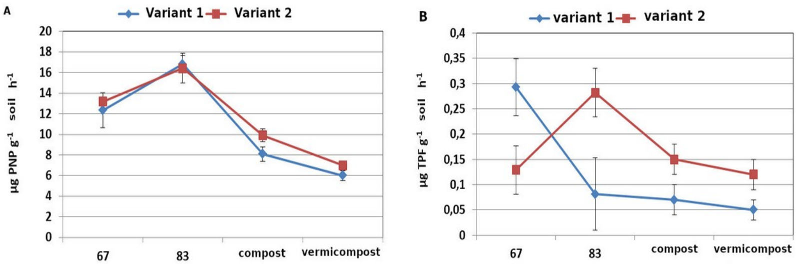

Figure 18 *β-glucosidase (A) and dehydrogenase (B) expression*

Several changes related to the concentration of the studied heavy metals into the final products, composts, and vermicomposts, had occurred during the processes (*fig.19*). Since the concentrations of heavy metals into the used biodegradable wastes are significantly lower than sludge, their combination reduced their content at composts. At the final composts of both treatments, a significant decrease of some heavy metals concentrations (Cd, Zn) was observed compared with those established into the input raw materials.

The biodegradation processes lead to a significant reduction of the waste volume, which increases the concentration of some heavy metals (Pb, Cu, Ni, Cr) into the composts. The compost and the vermicompost from treatment 2 showed higher concentrations of all metals than those at treatment 1. Regarding the *Ordinance* requirements, the compost from treatment 1, which contained lower sludge concentration, met the requirements for product *organic soil improver*. The compost of V-2 failed to meet the criteria for product *organic soil improver* at Cu content (445 $mg.kg^{-1}$), at the limit value of 400 $mg.kg^{-1}$ set at the *Ordinance*.

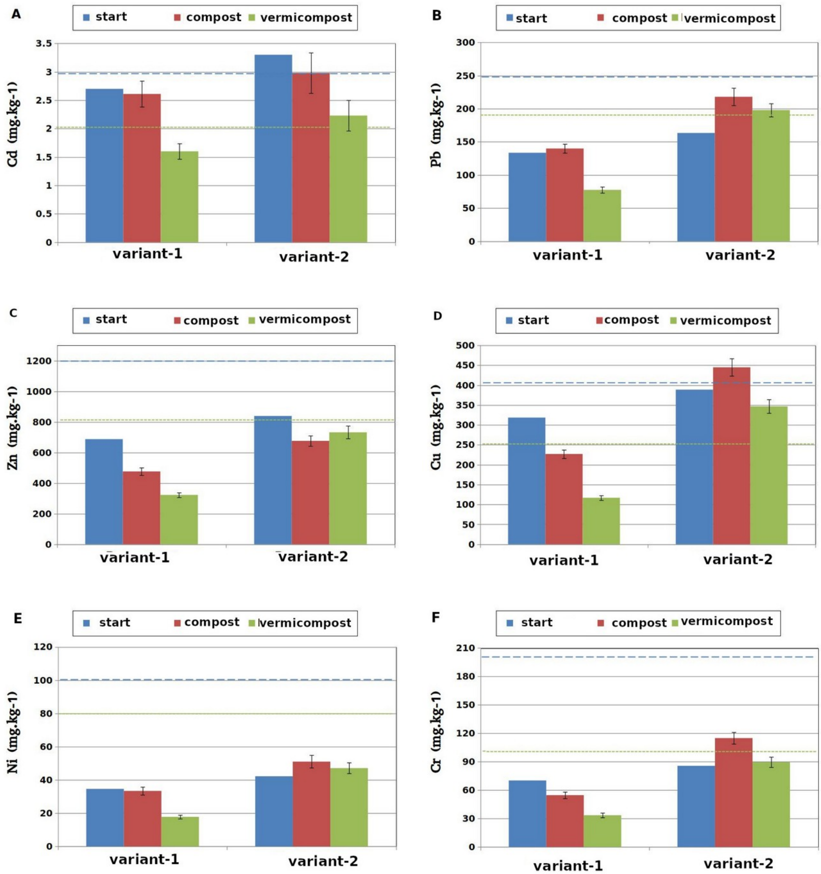

Figure 19 *Concentration of Cd (A), Pb (B), Zn (C), Cu (D), Ni (E), and Cr (F) into the materials, composts, and vermicomposts and standard error (n = 3)*

At vermicomposts of both treatments, a decrease at almost all metals compared to composts were observed (except for Zn content in vermicompost of V-2). This can be attributed to infiltrate leakage from the vermicomposting system due to precipitations or irrigations during the vermicomposting period.

Data of significant reduction of concentrations of Cd, Zn, Cu, Hg, Mn, As, and Pb during vermicomposting are widely present in the scientific literature and refer to possible accumulation into the earthworm tissues. In addition, the mixed population of *Eisenia fetida* and *Lumbricus rubellus* was better developed than at experiment 1 and experiment 2. This can be attributed to the improved suitability of the *composter system compared to the bed system* in terms of moisture retention.

After three months of vermicomposting, the volume of material had decreased by 1/3, although undecomposed particles of the wood chips were still observed in the material. The vermicompost from V-1 (a smaller amount of sludge) fully met the requirements for product *compost*. The vermicompost of V-2 failed to meet the requirements for product *compost* at Cu, Cd, and Pb indicators, but met the requirements for product *organic soil improver*.

The flow of the infiltrate, released from the composting piles, existed throughout the composting process but decreased during the second stage – the maturation. During the vermicomposting, several waterings were carried out to maintain the material's adequate moisture. The collection of resulting leachate was used to establish the flow of heavy metals leaving the system. The data are shown in *Table 1*.

Table 1 *Concentrations of some elements into the leachate during the vermicomposting process. Data show the mean values and standard error (n = 3)*

Eelement (μg.g^{-1})	Treatment 1	Treatment 2
Cadmium	0,05±0,004	0,08±0,01
Lead	1,6±0,1	4,1±0,3
Zinc	2,4±0,3	6,1±0,8
Copper	1,8±0,1	2,5±0,5
Nickel	0,1±0,01	0,3±0,02
Chrome	0,1±0,02	0,4±0,03

The most significant concentrations in the leachate were those of Pb, Zn, and Cu, while the lowest were of Cd, Ni, and Cr. The highest concentrations of Pb, Zn, and Cu were found at V-2.

Table 2 *Heavy metals content into the earthworms at the end of the vermicomposting process. Data show the mean values and standard error (n = 3)*

Eelement (μg.g^{-1})	Treatment 1	Treatment 2
Cadmium	0,2±0,01	0,25±0,02
Lead	1,1±0,1	0,9±0,1
Zinc	31±3,2	49,1±3,1
Copper	7,5±0,5	11,6±2,1
Nickel	1,4±0,1	2,0±0,17
Chrome	0,95±0,12	1,4±0,21

At the end of the vermicomposting were examined the concentrations of heavy metals into the earthworm tissues (*Table 2*). The most significant accumulated concentrations were of metals Zn and Cu, but the presence of Cd, Pb, Ni, and Cr was also observed. A statistically proven difference was found between the two treatments of the experiment in favor of treatment 2 at Cd, Zn, Cu, Ni, and Cr, while more Pb was found at treatment 1.

Table 3 *Pathogenic MO into the used WWTP sludge, composts, and vermicomposts of experiment № 3*

Indicator	**WWTP sludge**	**Compost**		**Vermicompost**	
		Treatment - 1	**Treatment - 2**	**Treatment - 1**	**Treatment - 2**
Salmonella sp.	presence	absence	absence	absence	absence
E. coli – титър	0,001	1	1	1	1
Cl. perfringens	0,001	0,001	0,001	0,01	0,01

Table 3 presents the content of pathogenic microorganisms into the used WWTP sludge and at the final compost and vermicompost. One of the most important indicators is the presence or absence of *Salmonella sp.* It was found that *Salmonella* sp. presented in a 20 g sample of sludge, while the bacterium was not found in the composts and vermicomposts samples.

Escherichia coli titer is another indicator showing the minimum volume in which this bacterium is presented. It represents the amount of water (cm^3/g) in which the bacterium is found. The *E. coli* cells were observed in 0,001 g of sludge. After biological treatments, in all composts and vermicomposts, the minimum amount in which cells were found is 1 g.

Clostridium perfringens was found in 0,001 g of sludge, as well as in both treatments. The presence of this pathogen can be explained by its resistance to adverse conditions because of its ability to form endospores. The incidence of *Cl. perfringens* was above the limit (1 cfu/0,001 g = 1000 cfu/g) in the final compost. After vermicomposting, 100 cfu of *Cl. perfringens* were detected in one gram of vermicompost (1 cfu / 0,01 g = 100 cfu / 1 g).

4.4. Biotests of composts and vermicomposts under controlled conditions

4.4.1. Composts and vermicomposts obtained from WWTPs sludge and biodegradable wastes from agriculture

The green pea plants (*Pisum sativum* L.) growth in the presence of compost is shown in *figure 20*. Statistical analysis by the LSD method showed no proven difference at α=0,05. In a comparison of the average values, according to the requirements of *Ordinance,* stem length of plants in V-1 and V-3 had a statistically proven difference with the control (standard error, 21,2% and 21,6%).

LSD analysis of variance showed the presence of four groups based on root length results. There was no statistically proven difference between the control (growth in soil) and the other treatments without V-3. The longest root length was found in V-3. The observed values of pea root length between the control, V-2 and V-4 are within the permissible deviation according to the *Ordinance* (up to 10%) of 6,6% and 5,3%, respectively. It can be concluded that the use of soil to composts mixture 1:1 (v/v) obtained at experiment №1 does suppress the development of pea plants. The use of compost obtained with 40% sludge led to the best growth of the aboveground part (21,6%) and the roots (25%).

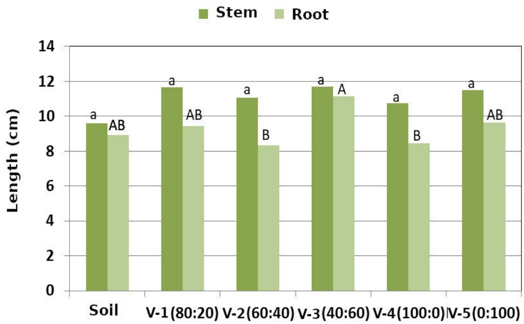

Figure 20 *Stems and roots lengths of peas growing in soil and compost 1:1 (v:v) from experiment №1. There was a statistically proven difference between the different letters for the different variables based on the LSD method of ANOVA at α = 0,05*

At the watercress seeds, biotest with vermicompost aqueous extracts was observed 100% germinated seeds at the control (distilled water.) The same results were reported for the treatments incubated with vermicompost extracts from V-2 (60:40) and V-3 (40:60). The germination of seeds incubated with garden soil extract and those with vermicompost extract of V-1 (80:20) and V-5 (0:100) was found to be 95%. The lowest germination of watercress seeds (90%) was observed at the seeds incubated with vermicompost extract from V-4 (100: 0).

The incubation of watercress seeds at different treatments for five days led to differences in growth expressed at the aboveground part and the root length of the plants. Based on the length of the watercress stem data, five groups of results were formed, in which the average values did not differ significantly from each other (*fig.21*). The shortest stems were observed at plants incubated with d.H_2O, followed by those with soil extract. The strongest growth was found at the plants incubated with vermicompost extract from V-1. Despite these variations, no significant difference was observed between plants of V-1, V-4, and V-5, while compared to all other V-1 showed the best, statistically proven results.

As a result of the analysis of variance of the root length data, four groups were formed, in which the average values did not differ significantly from each other. The longest root length was showed the plants incubated with vermicompost extract from V-1, followed by V-3, V-2, and the control. However, the analysis by the LSD method at α = 0,05 proved the absence of a significant difference between them. A significant difference was found between the above-mentioned treatments and the treatment with soil extract (the shortest root length). A significant difference was also observed between the plants of V-1 and those of V-4 and V-5. As a result of the above, it can be concluded that the aqueous extract of vermicomposts of all treatments does not have a suppressive effect, both on the seeds germination and the development of the aboveground part and root system of watercress.

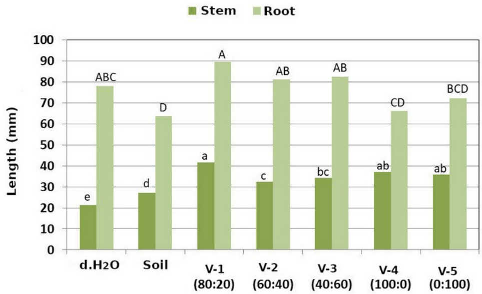

Figure 21 *Average of stems and roots length of watercress plants incubated with water, soil or vermicompost extracts of experiment №1. There was a statistically proven difference between the different letters for the different variables based on the LSD method of ANOVA (α=0,05).*

4.4.2. Composts and vermicomposts obtained from WWTPs sludge and biodegradable wastes from landscaping activities

The growth data of green peas plants with soil and compost substrate (1:1) is shown at *figure 22*. The comparison between the control (soil) and the two treatments with compost showed no inhibition at pea growth, as a result of compost presence. Plants grown with V-1 compost had 11% and those with V-2 compost, 15% longer stems than the control. Regarding the root length indicator, was observed a slight lag in growth compared to control plants. From a statistical point of view, no proven difference was found between the plants grown with soil and those of the two treatments, both in terms of aboveground part and root lengths of green pea plants.

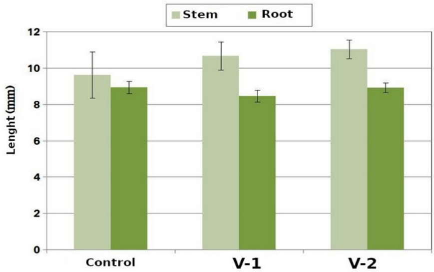

Figure 22 *Average length of the stems and roots of pea growing with soil (control) and soil and compost substrate 1:1 (v:v) of experiment №2*

The incubation of watercress seeds with the vermicompost aqueous extract of both treatments also showed no phytotoxic effect on the development of the aboveground part and the root system of the plants (*fig.23*). The average length of the aboveground part of V-1 plants exceeded control by 58% and V-2 by 53%. Similar results were obtained for plants incubated with aqueous soil extract, where V-1 plants exceeded them by 24% and V-2 by 20%. The plant roots of control (d.H_2O) showed almost the same values compared to V-1 and V-2, while compared with those incubated with aqueous soil extract, the increase was by 23% (V-1) and by 27% (V-2). Therefore, the aqueous extract from vermicomposts did not suppress, on the contrary, increased the growth of watercress plants. Better aboveground growth was observed at plants incubated with V-1 aqueous vermicompost extract, while roots were better developed at V-2.

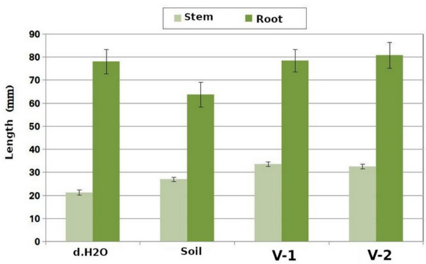

Figure 23 *Average aboveground parts and roots length of watercress plants incubated with d.H2O, soil, or vermicompost aqueous extracts of experiment №2*

4.4.3. Composts and vermicomposts obtained from WWTPs sludge in a combined approach

100% germinated watercress seeds were observed at the d.H_2O (control). The same result was found for seeds incubated with vermicompost aqueous extracts from treatment 1 and treatment 2. The germination of seeds incubated with extract of garden soil and those incubated with compost aqueous extract from treatment 1 was found to be 95%. The lowest germination was observed at seeds incubated with compost aqueous extract of treatment 2, 90%. The results showed that the seeds incubated with vermicompost aqueous extracts had higher germination than those incubated with compost aqueous extract of the same treatment. Germination of seeds incubated with vermicompost extract of treatment 1 is higher by 5,3%, and treatment 2 by 11%.

Regarding the length of the aboveground part of watercress plants (*fig.24*), the lowest values were observed at the control (d.H_2O), followed by the plants incubated with extract of garden soil. The other treatments showed no statistically significant differences, but their results were proven to be higher than those of the two controls. As a conclusion of the mentioned-above, the aqueous extract of composts and vermicomposts in both treatments did not significantly affect seed germination and development of the aboveground part of young watercress plants. Thus, they met the requirements according to the *Ordinance for separate collection of biowaste and treatment of biodegradable wastes* from 2017.

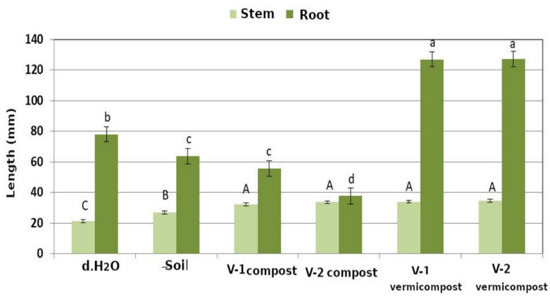

Figure 24 *Average of stems and roots length of watercress plants incubated with d.H_2O, soil, compost, or vermicompost aqueous extracts. Mean values and standard error (n=20) are shown. The different uppercase and lowercase letters indicate existing differences between the treatments (one-way analysis of variance, $p \leq 0,05$)*

The lowest average root growth was found at the plants incubated with aqueous extract of composts of the two treatments, whose growth was significantly lower than the control (51,7% and 28,5%). Unlike the above, the growth of watercress roots at treatments incubated with aqueous extracts of vermicomposts was more considerable than the other treatments, including the controls. The average root lengths were 62,6% and 63%, respectively longer than the root length of control (d. H_2O) and by 99,1% and 99,6%, respectively, compared to the soil extract treatment.

4.5. Application of the obtained composts and vermicomposts in the cultivation of tomato and pepper plants at field conditions

4.5.1. Vermicomposts obtained from WWTPs sludge and biodegradable wastes from agriculture

The field condition biotests provide the most realistic data on the applicability of studied composts and vermicomposts. During the tomatoes (*Solanum lycopersicum* L.) cultivation, the use of vermicomposts from all treatments of experiment №1, at the two studied concentrations, does not inhibit and even stimulates the development of both the root system and the aboveground part of the plants *(fig.25)*. All treatments showed values exceeding the control (growth with garden soil). In terms of stem length and leaf mass, the best developed at the two studied concentrations were the plants of V-5 (0:100). In terms of root length, the most vigorous growth was found at V-3 (40:60).

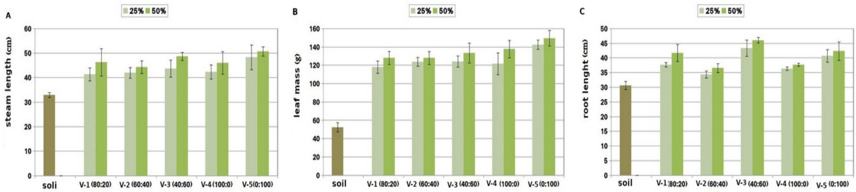

Figure 25 *Stem length (A), leaf weight (B), and root length (C) at tomato plants grown with soil or with soil and vermicomposts mixtures at the concentration of 25% and 50% (v:v) from experiment №1*

During the pepper biotests (*Capsicum annuum* L.), the use of vermicomposts in all treatments and concentrations, did not inhibit but even increase the growth of both the root system and the aboveground part of plants (*fig.26*). As with the tomatoes, all treatments, at both studied concentrations, showed results exceeding the control. The longest average stem length and also the largest average leaf mass were found at V-3 plants (40:60), but the longest roots were observed at V-1 plants (80:20).

In addition, the use of vermicompost of all treatments in the experiment №1 did not suppress but even stimulated the fruit formation of pepper plants.

The number of fruits formed at treatments with vermicomposts exceeded those grown without vermicompost (results not shown).

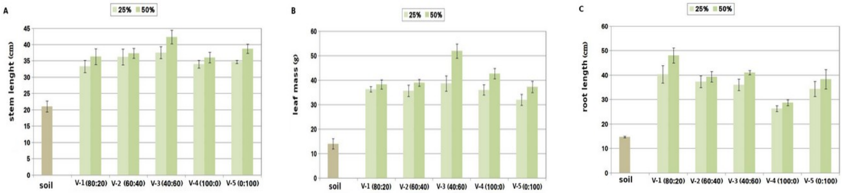

Figure 26 *The average stem length (A), leaf weight (B), and average root length (C) of pepper plants grown with soil or with soil and vermicompost mixture at 25% and 50% (v:v) from experiment №1*

Table 4 shows the Cu content into the aboveground part of the tomato plants. An increased Cu content was observed as a result of the incremented sludge concentration in the input materials. However, the plants grown with 50% vermicompost and 25% had similar Cu concentrations accumulated in their aboveground parts. A relatively good correlation was found between the concentration of Cu at the vermicomposts and at the plants when applying 50% vermicompost (r^2=0,80), while at 25%, the correlation was weaker, r^2=0,56. The Cu accumulation at the sludge-free treatment (V-5) had values practically equal to those at the control treatment.

Table 4 *Cu content into the aboveground parts of tomatoes, grown with vermicomposts from experiment №1. The data shows the average of the three plants. The standard error is within 5%*

Cu ($mg.kg^{-1}$)	**Control (soil)**	**V-1 (80:20)**	**V-2 (60:40)**	**V-3 (40:60)**	**V-4 (100:0)**	**V-5 (0:100)**
25%	68,2	171	172,1	184,3	174,1	60,3
50%		177,1	165,7	178,0	220,3	74,5

Table 5 *Zn content into the aboveground parts of tomatoes grown with vermicomposts from experiment №1. The data shows the average of the three plants. The standard error is within 5%*

Zn ($mg.kg^{-1}$)	**Control (soil)**	**V-1 (80:20)**	**V-2 (60:40)**	**V-3 (40:60)**	**V-4 (100:0)**	**V-5 (0:100)**
25%	36,0	48,1	60,2	66,3	64,1	50,7
50%		56,1	104,4	110,2	78,1	54,6

Although the Zn content at the vermicomposts was significantly higher than the Cu, its accumulation into the plant tissues was lower in absolute terms. At plants grown with 25% vermicompost, the highest concentration of Zn was found at V-3, V-4, and V-2. The lowest concentration was obserwed in the plants of V-1 and V-5 (*Table 5*).

In general, the differences between the treatments with WWTP sludge were not significant except for V-1, in which the Zn concentration was lower. The plants from the control (soil) treatment accumulated at least zinc, 36 $mg.kg^{-1}$. The Zn concentration in the "sludge-free" treatment (V-5) reached 50,7 $mg.kg^{-1}$, so the application of the product contributes about 41% to the accumulation of the metal. All plants grown at 50% vermicompost concentrations accumulated more Zn into the aboveground parts than garden soil plants (control). Compared to the control, the application of vermicomposts contributed significantly at Zn concentration enhancement. In this sense, the Zn is an essential element for plant development.

The accumulation of Cu into the tomatoes resulted in relatively high bioconcentration factor (BCF) values at the different treatments of experiment №1 (*fig.27A*). Doubling the concentration of vermicomposts in the tomato pots did not lead to a proportional increase in BCF. It is noteworthy that when using 25% vermicompost, lower WWTP sludge concentrations led to higher accumulation values compared to 50% vermicompost.

The bioconcentration factor of Zn into the tomato plants was lower in absolute value compared to the Cu. There was a tendency to increase the BCF of Zn by increasing the manure concentration (*fig.27B*).

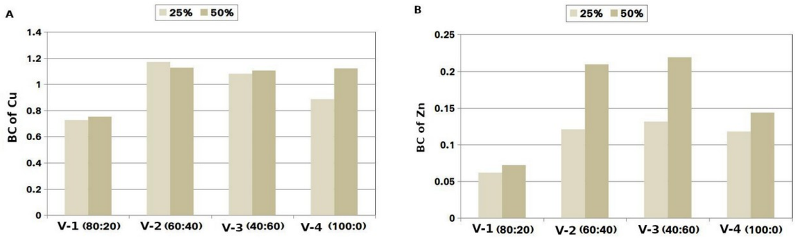

Figure 27 *Bioconcentration factor of Cu (A) and Zn (B) at tomato plants in both concentrations of vermicompost 25% and 50% at experiment №1*

4.5.2. Vermicomposts obtained from WWTPs sludge and biodegradable wastes from the landscape activities

The average stem length and mass leaf weight of the tomatoes grown at vermicompost mixture from both treatments exceeded the control plants (fig.28) significantly. At both treatments, the plants grown at 25% concentrations of vermicompost had average longer stems compared to those grown at 50% vermicompost. On the other hand, the 50% vermicompost concentrations of both treatments had a heavier average weight of the leaf mass than those of 25% concentrations.

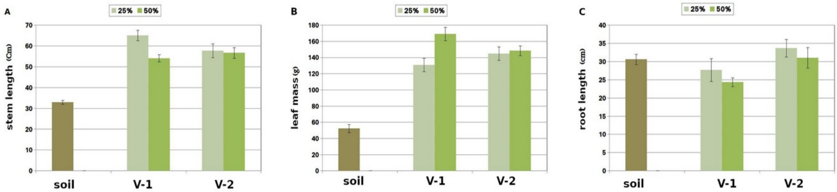

Figure 28 *Average stem length (A), leaf weight (B) and root length (C) of tomato plants at the treatments of experiment №2*

At both V-1 concentrations, there was some lag at average root length compared to control plants (*fig.28C*). Also, at both treatments of experiment №2, tomato plants grown at 25% vermicompost concentrations had, on average, longer roots than those at 50% concentrations. However, both concentrations met the requirements for indicators stem length, leaf mass, and root length set at the *Ordinance.*

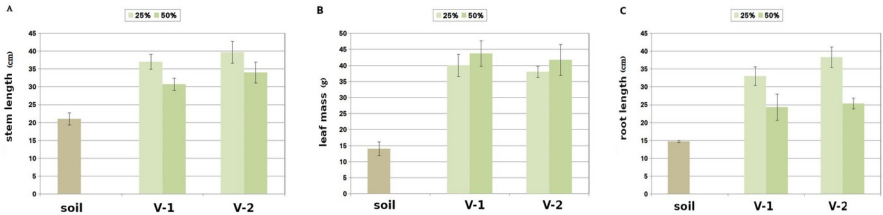

Figure 29 *Average stem length (A), leaf weight (B) and root length (C) of pepper plants at the treatments of experiment №2*

Regarding the development of the pepper plants can be concluded that the use of vermicompost at two treatments, at the two studied concentrations, does not inhibit but even stimulates the development of the root system and the aboveground part of the plants (*fig.29*). Both treatments, at the two concentrations, showed results exceeding the control. Better developed plants in terms of average stem length had V-2, while better-developed leaf mass was found at both V-1 concentrations. Plants grown at 25% vermicompost of both treatments had a better-developed root system than the others. The number of generative organs (fruits) of pepper plants grown at a mixture with vermicompost, at both tested concentrations, was higher than those grown at the control. The addition of vermicompost to the soil, at the studied concentrations, did not lead to suppression and even stimulated fruit formation.

As well as at experiment №1, with a higher average number of fruits were the plants grown with vermicompost from the treatment with less sludge in its composition (V-2).

The main elements accumulated into the leaf mass of tomato plants at experiment №2 were Cu and Zn (*Table 6*). The analyzes showed that only 50% vermicompost concentration at V-1 had higher accumulation of Cu into the aboveground part of the plants (over three times higher). At the same time, the values were very similar to the control treatment at the other treatments. The same trends as the Cu, were observed at the Zn accumulation. The highest concentration of Zn into the aboveground parts of the tomato pants was found at 50% vermicompost from V-1, which exceeded by 65,3% the control concentration.

In comparison, the 25% vermicompost of the same treatment showed a lower concentration. The plants grown at V-2 vermicompost mixture (25% and 50%) showed a slightly higher accumulation of Zn than the control plants. The Zn bioconcentration factor into the aboveground parts of tomato plants was lower at absolute value than Cu (results not shown).

Table 6 *Heavy metals content into the aboveground parts of tomatoes grown at vermicompost mixtures of experient №2*

Elements ($mg.kg^{-1}$)	**Control (soil)**	**V-1**		**V-2**	
		25 %	**50 %**	**25 %**	**50 %**
Cu	46,1	42	198	46	50
Zn	37	30	62	44	52

4.5.3. Composts and vermicomposts obtained from sludge from WWTPs in a combined approach

Some of the most critical parameters that give an objective assessment of plant growth are the stem and root lengths and the mass leaf weight of the test plants. Best values for all these parameters were observed at 50% vermicompost of treatment 1, followed by plants grown at 50% vermicompost of treatment 2 (*fig. 30*). Regarding all biometric parameters, the most backward at their development were the plants from the control (soil without compost), which could be attributed to the lower nutrient supply at this treatment. The use of vermicompost had a better effect on the tomato plant's growth and development compared to compost, and the difference varied widely at different treatments and concentrations. The lower concentration used at both treatments was insufficient to ensure plant nutrition during the vegetation period.

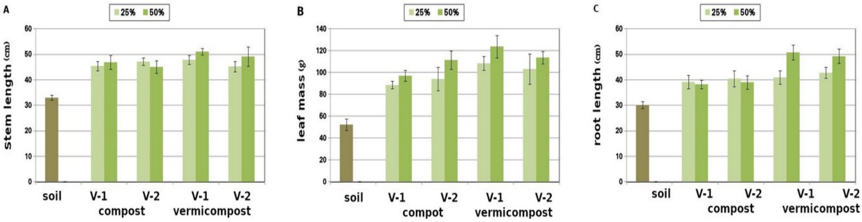

Figure 30 *Stem length (A), leaf weight (B) and root length (C) at tomatoes grown with soil or a soil, compost or vermicompost at 25% and 50% (v:v) mixture of experiment №3*

The pepper biometric results showed that compost and vermicompost didn't inhibit and even stimulate the root system development and the aboveground part of test plants (*fig.31*). The plants with the longest stems, roots, and the best-developed leaf mass were grown at a 50% concentration of vermicompost from treatment 2. The lowest average values at all measured parameters were found at the control plants, attributed to the lower nutrient content in the used soil. The obtained results at both crops meet the requirements for use in agriculture according to the *Ordinance* of 2017.

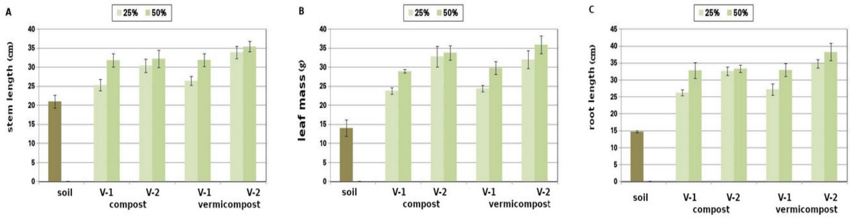

Figure 31 *Average stem length (A), average leaf weight (B), and average root length (C) at pepper plants grown with soil, compost, or vermicompost at 25% and 50% (v:v)*

Regarding the fruits formation of pepper plants, it was found that the average number of the obtained fruits exceeds those at the control plants at both treatments and tested concentrations of compost or vermicompost. In contrast with the results obtained at experiments №1 and №2, in which more fruits were formed of the treatments with less sludge, at experiment №3, more fruits were produced by the plants grown at the treatment with higher sludge concentration (V-2).

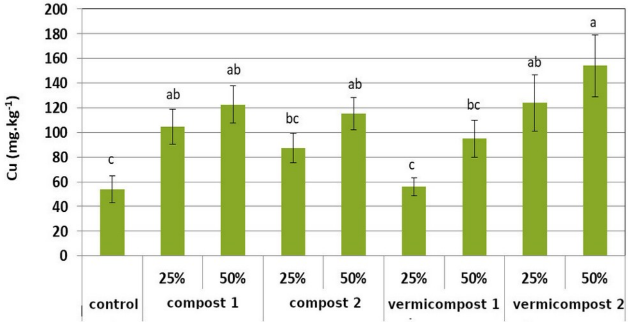

Figure 32 *Cu content of the aboveground part of tomatoes grown at 25% or 50% compost or vermicomposts in treatment 1 or 2. The data show the mean value and the standard error (n=3). Different letters show proven differences between the treatments in the one-way analysis of variance (p≤0,05)*

The analysis of Cu content at the aboveground part of tomato plants showed a certain dependence betwen the accumulation of metal and its content at the compost and vermicompost (*fig.32*). Increasing Cu content at compost or vermicompost due to the increased concentration of sludge into the input materials increases its accumulation in the plants. However, the significant difference in Cu concentration between compost 1 and 2 did not lead to statistically proven differences in the concentration of this element in the plant tissues. Although, these values were higher than the concentration found at the control plants.

Significant differences in the Cu accumulation were found at the plants grown with vermicomposts due to the different concentrations in the product. The Cu accumulated into the plants grown with 25% vermicompost of treatment 1 was similar to those at the control. Although the concentration was lower at these plants than the concentration found in the plants grown with 50% vermicompost, no statistically proven differences were found. The statistically proven difference was observed at plants grown in 25% and 50% vermicompost of treatment 2.

The accumulation of elements in plant shoots depends on their concentration and availability in the soil. In this sense, the content of Cu at the composts and vermicomposts impacted the accumulation into the plants. Cu's bioconcentration factor (BCF) was the lowest at plants grown with compost of treatment 2 (*fig. 33*), despite the higher metal concentration of treatment 2, if compared to the compost of treatment 1. This is probably due to the reduced availability of the elements in the compost of V-2 due to higher concentration of biopolymers and incompleted composting. Similar bioconcentration factor values were observed at plants grown at the presence of 25% compost of treatment 1 and both concentrations of vermicomposts of treatment 1, while the concentration of 50% BCF was about 36% lower. The lowest BCFs of Cu were calculated at the treatment 2 products. The reduction of the BCFs was observed at the treatments with 50% compost and vermicompost. Despite the lowest concentration of Cu into the garden soil, the highest BCF was found at the control plants. From the mentioned above, it can be concluded that the higher concentrations of Cu in compost or vermicompost of treatment 2 resulted in a lower accumulation into the leaf mass of the test plants compared to treatment 1.

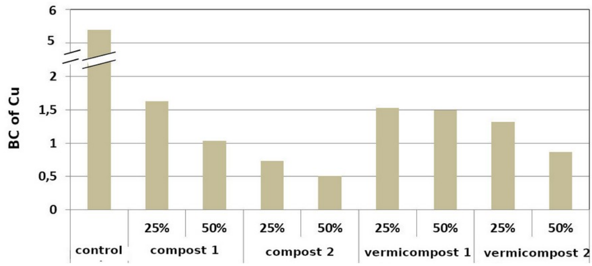

Figure 33 *Bioconcentration factor of Cu for the tomato plants of experiment №3*

The Zn content into the compost of treatment 2 was 42% higher than at treatment 1. This variance did not lead to statistically significant differences at the element concentrations into the tomato leaves at both used concentrations (*fig.34*). A significant difference was observed in the concentration of Zn between both vermicompost treatments. Despite of that, it did not lead to statistically significant differences in the accumulation of the element at p≤0,05. Differences were observed at Zn content of plants grown with 50% vermicompost of treatment 2 and the plants of all other treatments.

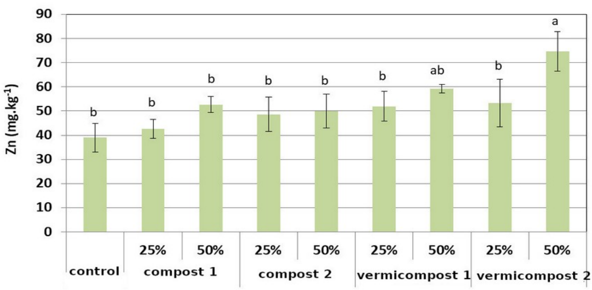

Figure 34 *Zn content into the aboveground part of tomatoes grown at 25% or 50% compost or vermicompost of treatments 1 and 2. The data show the mean value and the standard error (n=3). The different letters show proven differences between the treatments in the one-way analysis of variance (p≤0,05)*

The bioconcentration factor values of Zn followed the same trends as at Cu (*fig.35*). The higher concentration of Zn at the compost and especially at the vermicompost of treatment 2 resulted in a lower BCF of Zn. At vermicompost of treatment 1, BCF was significantly higher than in the compost, while at treatment 2 such a difference was found only at a higher concentration. Compost and vermicompost at both treatments, the higher concentration of the products in soil, resulted in lower BCF of Zn.

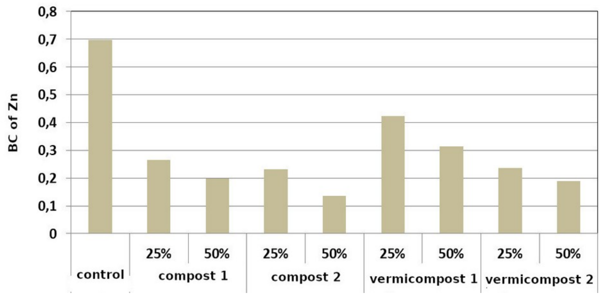

Figure 35 *Bioaccumulation coefficient of Zn at tomato plants at different treatments of experiment №3*

4.6. The compliance analysis of the composts and vermicomposts quality with the requirements of the *Ordinance for separate collection of biowastes and treatment of biodegradable wastes* from 2017

The *Ordinance for separate collection of biowastes and treatment of biodegradable wastes* is the primary document that regulates the permissible biowastes for compost production (within the meaning of this Ordinance, vermicompost also falls into this category, Tables A1-1, and A1-2). It also explicitly describes the quality requirements of the final product *compost* or product *organic soil improver*.

Table 7 summarizes results at the compliance of composts and vermicomposts with the requirements of the *Ordinance* of 2017. At all three experiments, vermicomposts meet the requirements for the final product *compost*. In the composts obtained at experiments 1 and 2, the discrepancy is associated with increased electrical conductivity and reduced germination, which indicates an incomplete composting process and insufficient maturity of the composts. The composts from experiment №3 met the requirements, with a particular drawback, due to the failure to fully achieve the required temperature regime. However, that has complied with pathogen requirements.

Table 7 *Compliance of some parameters in composts and vermicomposts from different experiments with the requirements of the regulatory framework*

Experiments/ Parameters	**Experiment 1: WWTO sludge +BWFA**	**Experiment 2: WWTO sludge +BWFLA**	**Experiment 3: Combined approach**
Temperature requirements	yes	yes	partially
Organic matter	yes	no	yes
Moisture	yes	yes	yes
Electrical conductivity	*compost*: partially *vermicompost*:yes	*compost*:no *vermicompost*:yes	*compost*: yes *vermicompost*: yes
Plant reaction/ growth test	germination *compost*: no germination *vermicompost*: yes *growth*: yes	germination *compost*: no germination *vermicompost*: yes *growth*: yes	germination *compost*: yes germination *vermicompost*: yes *growth*: yes
Pathogens indicator	*Salmonella*: yes *E. coli*: yes *Cl. perfringens*: yes	*Salmonella*: yes *E. coli*: yes *Cl. perfringens*: yes	*Salmonella*: yes *E. coli*: yes *Cl. perfringens*: yes

* *BWFA - biodegradable wastes from agriculture; BWFLA - biodegradable wastes from landscape activities*

Important parameters for the quality of the final products (composts and vermicomposts) are the heavy metals concentrations, as these elements are often present in the WWTPs sludge. The maximum permissible concentrations of heavy metals are shown in Table A2-1 and A2-3 of the *Ordinance*, 2017. In some cases, one product fails to meet the requirements for product *compost* but meets those for *organic soil improver*. In contrast, *compost* can be used both in agriculture and horticulture. For reclamation of disturbed areas, the organic soil improver is supposed to be used in agriculture only for crops, not human food or animal fodder. The product *organic soil improver* is suitable mainly for landscaping activities and reclamation disturbed areas or landfills.

The analysis results of the composts and vermicomposts at experiment 1 (*Table 8*) showed non-compliance with the requirements at composts of V-1 (Cd, Cr, Cu, and Pb) and V-4 (Cr and Pb), which is attributed to the higher concentration of WWTP sludge into the composting piles of these treatments. These concentrations decreased during vermicomposting as a result of periodic irrigations in order to maintain an adequate moisture of 70-75%. As a result, the vermicomposts of all treatments comply with the requirements, aside from the Pb concentration at V-1. In this sense, the two products of biological treatments at experiment 2 met the heavy metal concentration requirements, aside from the Cd at V-1. At the third experiment, in which biodegradable wastes from agriculture and landscape activities were used, a particular discrepancy was observed at the accumulated concentrations. This mainly was the case of Cd concentration at the composts from both treatments and with Cr, Cu, and Pb at treatment 2. Vermicomposts showed compliance, aside from Cd and Cu in treatment 2.

Table 8 *Compliance of heavy metals content at composts and vermicomposts with the requirements of the regulatory framework (until 2020)*

Experiment Parameters	**Experiment 1: sludge +BWFA**	**Experiment 2: sludge +BWFLA**	**Experiment 3: Combined approach**
Cd	*compost*: without V-1 *vermicompost*: Yes	*compost*: V- 2 *vermicompost*: V-2	*compost*: No *vermicompost*: V-1
Cr	*compost*: without V-1, and V-4 *vermicompost*: yes	*compost*: Yes *vermicompost*:Yes	*compost*: V-1 *vermicompost*: Yes
Cu	*compost*: without V-1 *vermicompost*: Yes	*compost*: Yes *vermicompost*:Yes	*compost* V-1 *vermicompost* V-1
Hg	-	-	-
Ni	*compost*: Yes *vermicompost*:Yes	*compost*: Yes *vermicompost*: Yes	*compost*: Yes *vermicompost*: Yes
Pb	*compost*: without V-1 and V-4 *vermicompost*: without V-1	*compost*: Yes *vermicompost*: Yes	*compost*: V-1 *vermicompost*: Yes
Zn	*compost*: Yes *vermicompost*:Yes	*compost*: Yes *vermicompost*:Yes	*compost*: Yes *vermicompost*:Yes

4.7. Implementation of the sludge recycling technology at WWTP - Hisarya, WWTP - Sopot and WWTP - Karlovo

Based on the results and conclusions of the present thesis, during Year 2017, along with Water and Sanitation – Plovdiv, a pilot project was launched to recycle sludge with other biodegradable wastes at their formation (WWTP - Hisarya). For the project purposes was used aerobic composting method followed by vermicomposting. The biodegradable wastes were obtained from the landscaping activities around the city of Hisarya. From 2011 until the project beginning in May 2017, the sludge generated by the WWTP-Hisarya was landfilled at Karlovo landfill located at 30 km distance from the plant. The annual amount of generated WWTP sludge with code (19 08 05) is about 350-400 t. The vermicompost from the first batch (about 50 m^3) met the requirements for product "*compost* "according to the *Ordinance* of 2017. Currently, the technology is successfully implemented, and the entire amount of sludge generated of the WWTP - Hissarya is composted and vermicomposted. The product vermicompost is used as a complex fertilizer for the green areas of the plant.

During the Year 2018, the technology of composting, followed by vermicomposting of WWTP sludge, along with other biodegradable wastes, was implemented at the place of their formation, at the WWTP - Sopot and WWTP - Karlovo. Both WWTPs have concrete sites designed for the temporary storage of dewatered sludge. Until the project began, the generated WWTPs sludge of both WWTPs was landfilled at Karlovo landfill, the annual amount of sludge with code (19 08 05) from WWTP - Sopot is about 300 t, and from WWTP - Karlovo is about 1500 t. Gradually, the entire generated amount of sludge at WWTP - Sopot (about 300 t/year) and ½ of the amount formed at WWTP - Karlovo (about 750 t./year) were subjected for composting and vermicomposting. The project used biodegradable wastes from urban landscaping and agricultural activities (grass, dry leaves, wood chips, straw). The vermicompost of the first set batch at WWTP - Karlovo was about 60 m3, and at WWTP - Sopot about 50 m3. The product covered the requirements for product "*compost* "and product "*organic soil improver* "according to the *Ordinance* from 2017. The vermicompost was used for landscaping purposes.

Composting and vermicomposting of WWTP sludge at the place of their formation was an innovative approach for our state. As a result of these projects, the method was established that could be successfully used to solve the problem with WWTP sludge at already existing small and medium WWTPs and to be planned as a subsequent unit into projects for new treatment plants. This could lead to developing a comprehensive concept for solving the problem of WWTP sludge by an innovative and environmentally friendly approach.

V. CONCLUDING REMARKS

Composting and vermicomposting are the most affordable methods for converting biodegradable wastes into valuable products. Traditional thermophilic composting is the method most often used for the treatment of WWTPs sludge. Furthermore, the composting pile goes through a thermophilic stage (45-65 °C) of intensive biodegradation in which the different types of microorganisms generate heat and help disinfect the final product. Subsequently, the heterogeneous input raw materials are turned into a homogeneous humified matter during the cooling period. During the vermicomposting, worms were fragmenting the organic matter, stimulating microbial activity, intensifying the biodegradation and the humus formation. Composting combined with vermicomposting resulted in a sufficient level of disinfection of substrate shortened the organic matter's stabilization time. It resulted in a product with the desirable characteristics, faster than each of the processes carried out individually.

This type of combined treatment of WWTPs sludge has not been done so far in our state. It can offer a solution to an existing problem of wastes management, such as sludge utilization. It is known that composts and vermicomposts are products rich in nutrients, micro-and macroelements, which have a positive effect on the growth and development of both ornamental and crops. At the same time, the application of compost into the soil increases the concentration of organic matter, improves the structure, moisture retention capacity, and amplifies the reserve of elements essential for plant development. Because of these effects, the biotests with tomatoes and peppers showed much-improved plant growth when applying 25% or 50% compost or vermicompost, compared to the control. Despite the presence of various heavy metals into the WWTP sludges, only zinc and copper were found in the plant tissues in concentrations comparable to those common to the respective crops.

The composting of bio-wastes is a process of utilization and material recycling, with code R3 in Annex № 1 to § 1, item 11 at the Final Provisions of the Law on Waste Management. It supports the reduction of greenhouse gas emissions and has a significant contribution to implementing the recycling objectives under Art. 31, par. 1, item 2. At the same time, composting is part of a circular bioeconomy aimed to reduce the usage of natural resources, where "waste materials are processed into products, materials or substances for their original purpose or for other purposes". It also reduces the volume of biodegradable wastes several times, prevents landfilling, diminishes the means of transport and disposal. The result is a high-quality product, which can be used successfully as a safe fertilizer for urban landscaping activities or agriculture.

5.1. Conclusions

The following conclusions were made, based on the obtained results:

1. The composting technology followed by vermicomposting is a type of *ex-situ* bioremediation of WWTPs sludge. The second process increases the efficiency of the treatment and results in a quality product formation.

2. The optimal period required for obtaining quality vermicompost from WWTP sludge at an industrial scale is between 180 and 230 days. It can be reduced by adjusting the concentrations of WWTP sludge and the biodegradable wastes containing cellulose and lignin.

3. Using wood chips over 30% of the input materials can delay the composting, insufficient transformation of organic matter, and prevent the temperatures of 55-65 °C, which ensure disinfection of the substrate. Likewise, the presence of more than 50% WWTP sludge leads to the incomplete conversion of ammonium to nitrate nitrogen, which is a premise of lowering the obtained composts and vermicompost quality.

4. The loss of essential elements during the processes could be reduced through irrigation with the collected infiltrate, which is applicable in the case of low heavy metals concentration in WWTP sludge and could lead to an increase of the final product quality.

5. There is a positive correlation between the concentration of heavy metals in WWTP sludge and their concentration in the final products. Removing the infiltrate during composting, and vermicomposting could be a convenient approach for reducing their concentrations. Therefore, in this way is possible to be used WWTP sludge with higher initial values of heavy metals than those mentioned in *Annex № 2* of the *Ordinance*, *2017*.

6. Vermicomposting appears to be a necessary biological step after composting of WWTP sludge. It improves the final product quality, expressed as increased seed germination and reduced electrical conductivity, and significantly reduces pathogens concentrations (*Salmonella* sp., *Escherichia coli,* and *Clostridium perfringens*).

7. Composts and vermicomposts obtained of biodegradable wastes containing up to 50% WWTPs sludge fully meet the Ordinance requirements *on separate collection of biowaste and treatment of biodegradable wastes* from 2017.

8. Tomato and pepper plants grown on a soil medium with compost or vermicompost of 25% or 50% are better developed and form more biomass than garden soil medium. This is an indication of the good final result of the combined biological treatment.

9. Plants grown on a medium containing compost or vermicompost accumulate only the trace elements Cu and Zn of the heavy metals present in WWTPs sludge. Their concentrations are within the normal range for the respective crops.

10. The obtained composts and vermicomposts are rich in nutrients. They can be used successfully in agriculture, landscaping activities, restoring the nutrient balance of poor and infertile soils, and reclamation of disturbed areas.

5.2. Contributions

5.2.1. Scientific contributions

1. For the first time in Bulgaria, a comprehensive study of WWTPs sludge treatment is conducted employing composting and vermicomposting.

2. A model for WWTPs sludge utilization at agriculture, landscaping activities, and reclamation of disturbed areas has been created.

3. An approach for lowering the concentrations of the heavy metals from WWTPs sludge via joint treatment has been established so that the obtained final product meets the requirements according to the *Ordinance on separate collection of biowaste and treatment biodegradable wastes* from 2017.

4. A method for loss reduction of organogenic elements by returning the infiltrate back to the system, which is applicable in the case of low heavy metals concentration into the WWTPs sludge.

5.2.2. Scientific and applied contributions

1. The vermicomposting technology has been improved, replacing *beds* with wood composters.

2. The circular economy principles have been clearly demonstrated through the recycling of wastes and their conversion into the products compost and vermicompost, which could be used successfully in agriculture.

3. The developed technology for recycling WWTPs sludge, at the place of its formation, has been successfully implemented at WWTP - Hissarya, WWTP - Sopot, and WWTP - Karlovo. As a result of the mention above, 1050 t per year WWTP sludge is subjected to composting. The obtained *in-situ* vermicomposts meet the product requirements, according to the *Ordinance on separate collection of biowaste and treatment of biodegradable wastes* from 2017. They are used as a quality fertilizer for landscaping activities.

5.3. List of publications related to the dissertation

The obtained results from the present study have been reported at current scientific forums and published in scientific journals:

1. **D. Angelova**, St. Shilev, 2016. Evaluation of joint composting of WWTP sludge with biodegradable wastes from parks in order to meet the requirements for utilization in agriculture. Collection of reports at "Ecology and Health" June 9-10, 2016, pp. 429-434, ISSN 2367-9530, http://hst.bg/bulgarian/ (BG)

2. **Angelova D**., Shilev, S., Naydenov, M. 2016. Composting of sewage sludge at large scale for subsequent utilization in agriculture. In: (Filcheva, Stefanova, Ilieva eds). 4th Nat. conf. of BHSS. 8-10 Sept. 2016, Sofia, ISBN 978-619-90189-2-7, 285-295.

3. Shilev S., Azaizeh H., **Angelova D**. 2019. Biological treatment: a response to the accumulation of biosolids. pp. 149-178. In: Singh, D. P., Gupta, V. K., Prabha, R. (Eds.) Microbial Interventions in Agriculture and Environment, Vol. 2: Rhizosphere, Microbiome and Agroecology. Springer Singapure. Doi: 10.1007/978-981-13-8383-0.

4. **Angelova D**., S. Shilev S. 2021. Composting and vermicomposting of biosolids for utilization in agriculture. Journal of Environmental Protection and Ecology 22 (3), 1030-1039.