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PhD THESIS

ABSTRACT

RESEARCH CONCERNING THE INFLUENCE OF SLUDGES FROM URBAN WASTEWATER TREATMENT PLANTS WITHIN ALFALFA CULTURE

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INTRODUCTION

Romania increased urbanization, and increased demands on the European Community municipal waste recovery requires solutions for reintroduction into agricultural production systems city sludge. City sludge recycle a range of essential nutrients for plant growth, phosphate mineral reserves that are currently based phosphorus fertilizer preparation, improves the physical, chemical and biological soil properties, considered as a true biological treatment plant for municipal waste these products. Municipal sludge in agriculture capitalization is determined both by the growing lack of fertilizers and continuous increase of price, and the lack of manure due to lower livestock in Romania, while the soil requires increased amounts of fertilizer to obtain high yields, stable and efficient.

CHAPTER I
GENERAL NOTIONS ABOUT CITY SLUDGE

Quantities of sludge resulting from wastewater treatment plants are growing from year to year, requiring viable solutions for their neutralization.

In recent years, worldwide, has reached a critical level in the accumulation of large amounts of urban waste, which mainly have three major sources: industry, sewerage and sanitation, which are collected from treatment plants, household and street work the residues were collected from special points preliminary storage.

After some calculations made, it was estimated that each person produces 800 kg sewage sludge (95% water), or 25-40 kg of dry matter per year, with some slight variations between countries.
CHAPTER II
MAIN CHARACTERISTICS OF CITY SLUDGE

In determining their nutritional value, agronomic and commercial chemical characteristics of sludge were instrumental in knowing the state of "health", the level of harm and/or toxicity against various compounds. From the chemical point of view these materials are characterized by a high degree of heterogeneity, which is determined by the large number of compounds, their dynamic nature and broad intervals varying characteristic.

Although fertilizing value of slurry is generally recognized, its widespread use in agriculture faces some objections concerning sanitary issues and the presence of undesirable elements such as: heavy metal compounds, pesticides and some degradable organic substances.

CHAPTER III
MOLECULAR NITROGEN FIXING SYMBIOSIS

The concept of symbiosis with the lives of special bodies, whether beneficial or harmful effect on one or both partners of the association. The concept of symbiosis is a long-term coexistence of two (or more species) living in the vicinity and gain mutual benefits from their interaction. Symbiosis may occur in the interaction between two organisms or micro and macrosimbiot.

Concerns about the increased production of vegetable and animal protein, while intended to reduce the amount of manure produced by industry are primarily related to finding new opportunities for service offered leguminous plants; more judicious use of legume crops for solving protein deficit is based on the fact that some members of this family, made symbiosis with N₂-fixing bacteria, Rhizobium genus.
CHAPTER IV
THESIS OBJECTIVES

The main aim of the thesis is:

1. assessing the potential use of sludge from WWTP Tetarom III, Cluj-Napoca as fertilizers culture of alfalfa through a complex and precise methodology,
2. sludge influence on the main parameters of soil microbiology and enzymology for monitoring environmental effects.

To achieve the research under this theme were set a series of replacement apparatus aimed at:

1. **Influence of urban sludge on productivity and forage quality in alfalfa:**
   - Characterization of Chemically, the way in which the material used (sludge arising from wastewater treatment plant), corresponds to the technically Technical Regulations Order 344/2004 of the Ministry of Environment and Water;
   - Tolerance index calculation sludge from WWTP Tetarom III, Cluj-Napoca on alfalfa culture;
   - Evaluation the effect of sludge fertilization on the production of green mass, dry matter (DM) and the alfalfa forage quality in terms of the experimental field ecopedological the Boldut.

2. **Influence of sewage sludge on soil characteristics:**
   - Characterize the main physical, chemical and biological soil of the experimental field according to soil analysis methodology developed by the ICPA Bucharest;
   - Assessment of heavy metal accumulation in soil following the application of different doses of sludge from wastewater treatment plant in Cluj-Napoca Tetarom III.

3. **Influence of city sludge on microbiological indicators:**
   - Evaluation sludge fertilization effect on soil respiration and evaporation;
   - Evaluation of sludge fertilization effect on the amount of symbiotically fixed nitrogen;
   - Study morphological and tinctorial properties of nodule.
CHAPTER V
MATERIALS AND METHODS

Experiences aimed at achieving the objectives in this thesis were conducted both in the experimental field of the Boldut, and in the laboratories of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca laboratory discipline grassland and forage Culture and Microbiology Laboratory and the Department of Animal GxABT University of Liège.

The biological material used in our experiments was alfalfa (*Medicago sativa L.*) variety Madalina, variety created National Research Institute Fundulea (NIRD).

Alfalfa seeding was conducted in spring 2010 with SUP-29 tractor trailer at a depth of 1.5-2 cm U650 at a spacing of 12.5 cm. Also, sludge application was made in a single installment in spring 2010.

Norma seed was 20 kg / ha which was good ground cover and weeds that have sprung from alfalfa could not hinder basic culture.

The experiments were located after the latin rectangle method 4 repetitions with 8 variants randomized as follows: V1-control, V2 - 20 t/ha sludge, V3 - 30 t/ha sludge, V4 - 40 t/ha sludge, V5 - 60 t/ha sludge, V6 - 20 t/ha manure, V7 - 40 t/ha manure, V8 - 60 t/ha manure.

Experimental field was divided into 8 types with 4 repetitions, variant cation measuring 40 m² (8x5m). Variant 1 was fertilized, the version control and variants 2,3,4 and 5 were treated with city sludge at different doses and variants 6,7 and 8 were fertilized with manure from farms Boldut.

Sludge used comes from wastewater of Cluj-Napoca, fermented and dried prior metatank beds for 8 months with the following characteristics: 3.56% N, 2330 ppm P, 816 ppm K and neutral pH 7, 40.

Chemical analyzes of the sludge and soil profile characterization were performed at OSPA Cluj-Napoca. Variants were mowed hand when the plants have reached the desired phenophase and harvest each variant were weighed to determine the yield of green mass. For quality measurements made in the laboratory samples were taken harvest
100 g samples using a sampler, so they should be as homogeneous. Alongside these works were performed maintenance such as cleaning paths and removing weeds.

5.1. DETERMINATION OF DRY MATTER (DM)

Alfalfa samples obtained from experimental field bold in 2010-2011 were used for the determination of dry matter (DM). These samples were taken with mows performed to determine the mass production of green. In order to calculate the production of DM, harvested alfalfa samples were ground to die Grindomix-200, after which they were dried in an oven for 6 hours at 60°C. Samples were taken from all experimental variants and repetitions, each sample weighing 100 g after oven drying the samples were reweighed to calculate production of DM.

5.2. FEED QUALITY DETERMINATION

To determine alfalfa forage quality, as well as for determining the solids content samples were taken with mows performed to determine the mass production of green. Determination of alfalfa forage quality was performed by the classical method of analysis of feed quality for the following parameters: protein, cellulose, lignin, digestibility, ADF and NDF.

5.3. DETERMINATION PLANT HEIGHT

Plant waist measurement was performed directly in the experimental field using a meter garden, in phenophase of buds and in the phenophase of flowering and seed formation in phenophase prior to determining that green mass production.
5.4. DETERMINATION OF TOLERANCE

Tolerance index is the ratio between production in soil treated and untreated soil production in highlights impact of treatment on production levels.

5.5. DETERMINING THE INFLUENCE OF CITY SLUDGE UPON SOIL CHARACTERISTICS

In order to describe the genetic type of soil, samples were taken and analyzed in terms of the physical and chemical properties at the beginning of the experiment and during this year to determine the soil profile, color, texture, humus, compactness, neoformation and pH. Soil samples were taken from the following genetic horizons: Ap (0-20 cm), ATP (20-32 cm), 2Abk (32-48 cm), bB1k (48-73 cm), bB2k (73-92 cm), Ck (92-120 cm) and were analyzed to determine the physical characteristics of the soil in the laboratory of Soil and Agrochemical studies Office Cluj-Napoca (OSPA).

5.6. DETERMINATION OF SOIL RESPIRATION AND EVAPORATION

In our experiment from Boldut, for monitoring respiration and soil evaporation we used a closed dynamic system developed by U.S.A., firm PP System (CIRAS 2).

5.7. DETERMINATION OF SYMBIOTICALLY FIXED NITROGEN (NFB)
In our experiments from Boldut experimental field were determined as follows:
1. symbiotically fixed nitrogen from alfalfa, under use by mowing (NFBc);
2. daily rate of nitrogen fixation in alfalfa culture (RzNFB);
3. average annual rate of nitrogen fixation (RmaNFB) related to DM.

To calculate the amount of biologically fixed nitrogen was used regression method based on the production of DM, using the formula established in 2003 by CARLSSON and HUSS Danella. Thus the formula for determining symbiotically fixed nitrogen from alfalfa crop is as follows:

\[ NFBc = 0.013 \times DM \times 12.3, \]

where

NFBc is the amount of nitrogen fixed in the conditions of use by mowing,
DM is dry matter.

To determine the daily rate of nitrogen fixation in alfalfa culture we used the following formula:

\[ R_{zNFB} = \frac{NFB}{L\%} \]

where

NFB is biologically fixed nitrogen (kg / ha),

L\% is the proportion of alfalfa in floristic composition (which is 100\% for our variants).

To determine the annual average rate of nitrogen fixation compared to DM formula was used:

\[ R_{maNFB} = \frac{NFB}{DM} \]

where

NFB is biologically fixed nitrogen (kg / ha),

DM is the average annual amount of dry matter (t / ha).

5.8. DETERMINATION OF SOIL ACTIVITY SYMBIOTIC

For determining sewage sludge fertilization effect on soil microbiological parameters and Enzymology, with special reference to nitrogen fixing bacteria of the genus Rhizobium, we used the Gram method and differential method.

CHAPTER VI
RESULTS AND DISCUSSION

6.1. THE INFLUENCE OF CITY SLUDGE APPLICATION UPON PRODUCTIVITY AND QUALITY OF FORAGE AT ALFALFA

In 2010 the largest DM productions were recorded in the variants treated with 40 and 60 t / ha city sludge (respectively 9.16 and 9.10 t / ha DM with a difference of 1.69 and 1.63 t / ha DM compared to the control treatment). In the variants fertilized with manure, most production has been higher in the variant fertilized with 40 t / ha with a production of 8.88 t / ha DM showing a difference of 1.41 t / ha compared to the control fertilized.
At the same dose applied sludge 40t/ha and 40 t/ha manure, in I noticed an increase of production is more important for sludge (9.16 t DM/ha to 8.88 t DM/ha application of manure), situation persists in second year (11.63 t DM/ha application of 40 t/ha sludge and 11.32 t DM/ha application of 40 t/ha manure).

In 2010 we obtained very significant (in variants treated with 40, 60 t/ha sludge and 40 t/ha manure) to control and significantly distinct (in variants fertilized with 20, 30 t/ha sludge and 20, 60 t/ha manure) compared to the control. In 2011 the largest SU productions were recorded in the variants treated with 40 t/ha city sludge and 40 t/ha manure (respectively 11.64 and 11.33 t/ha DM with a difference of 3, respectively 27 and 2.96 t/ha DM compared to the control treatment). At these doses equivalent to 40 t/ha manure and sewage sludge to obtain a difference of 0.31 t/ha DM for variant treated with city sludge.

In 2011 in all experimental variants were obtained very significant production increases from unfertilized control variant, increases ranging from 2.18 t/ha DM (in variant treated with 20 t/ha city sludge) and 3.27 t/ha DM (the variant fertilized with 40 t/ha city sludge).

In determining forage quality resulting from crops sown in the experimental field of alfalfa in bold were obtained by dividing laboratory analytical samples were analyzed and organoleptically control, botanical, physical or chemical (crude protein, NDF content, content the ADF, lignin, digestibility). Each analysis was done by classical method and by NIR method. To determine forage quality samples were obtained from 100 g of alfalfa, which were homogenized and analyzed.

NIRS technique has become in recent years a method of quality forage widely used and is a very elegant and precise.

The mathematical model was built based PLS (Partial Least Squares) algorithm using MPLS (Modified PLS) using derivative preprocessing technique 1 to 5 points; SMOOTH 1 in 5 points and cross-validation (leave-one-out). The program used was ISI-Monitor V. 1.50.e.

Analyzing the experimental data obtained from fertilization with sludge urban wastewater treatment plants on alfalfa forage quality can be seen that, sludge fertilization does not result in changes in protein content, ADF, NDF, lignin, compared to unfertilized
alfalfa, and these quality parameters fall within the normal range for the species under study.

In addition, analyzing forage quality under fertilization with city sludge compared to organic fertilization with manure fertilization mud is found that does not change the chemical composition to feed alfalfa compared with no manure application, only exception: lower protein content compared to the control, the difference was only significant effect at doses of sludge of 60 t/ha.

The results obtained after the first year of sludge confirms data obtained by other researchers, showing that in the first year of application of sludge, chemical composition of alfalfa was less influenced.

If city sludge used to treat the sting and alfalfa from Tetarom III WWTP Cluj-Napoca not find heavy metal toxicities because existing sludge falls within the maximum limits.

Alfalfa treated with different doses of city sludge has a good tolerance as the ratio of production in soil treated with sludge from wastewater treatment and production in untreated soil exceeds value 1.

6.2. THE INFLUENCE OF CITY SLUDGE APPLICATION UPON SOIL CHARACTERISTICS

Genetic profile of soil in the experimental field profile Boldut is truncated by erosion surface, and for this reason can not make conclusive judgments pedogenesis.

However it seems that the Bt horizon is inherited from a soil that was subsequently covered by more recent material (proof would be discontinuity sand content). This question of the existence of a fine wind deposits in the Transylvanian Plain (at least in the Turda), which is not mentioned until now. B horizon clay content is significantly higher than the surface horizon to be considered Bt and give textural differentiation profile index of 1.2. Invasion of carbon appears to be linked partly as local condition (peak exposed to intense evaporation) and the forest vegetation in the Transylvanian Plain. It seems that contributed to recarbonatarea erosion profile.
By distributing sludge on farmland soil is used as a final purification step. Potential ground-handling mechanisms include: a purely mechanical restraint, biological oxidation, ion exchange, chemical precipitation, adsorption, absorption and assimilation by plants and living organisms. Thus, the soil is a biological station with all levels of treatment and its ability to process organic matter depends on its properties and climatic conditions. This means that it can not exceed a certain degree of waste products of the soil load without affecting its physical, chemical or biological weapons without affecting production or quality of production or to reduce or even cancel without scrubber role of soil (Dumitru, 1994).

After applying sludge from municipal wastewater treatment plant in Cluj-Napoca Tetarom III, our results confirm the literature data shows that the first year after sludge application and in the second year of soil physical traits are less influenced. For example, in our experiment at Boldut, there is changing soil texture (clay-loam) in any of the variants which have been applied to different doses of city sludge. Also, all experimental variants in the two years 2010-2011, the percentage of clay does not show major differences compared to the control unfertilized (51.69% in horizon Bt horizon I and 49.66 in the control variant to 50.92% to 50.14% in horizon I and Bt horizon in the variant fertilized with 40 t/ha city sludge).

The application once by three years, increasing doses of sewage sludge originating from the treatment plant Tetarom III, Cluj-Napoca, that doses of 20, 30, 40, 60 t/ha city sludge not cause changes in the physical (fine sand, coarse sand, dirt, clay and texture) of soil or first experimental year 2010, nor in the second experimental year 2011.

Application of city sludge from wastewater treatment plant in Cluj-Napoca Tetarom III on faeoziomul argic the experimental field of the sting did not lead to significant changes in soil chemical characteristics. Our results confirm the literature on the effect of sewage sludge on soil chemical characteristics. For example, in our experimentulu, it is changing the soil pH significantly in any of the variants with application of city sludge (8.07 and 8.39 in the horizon I Bt horizon in version control to 8.06 in the horizon I Bt horizon and 8.41 in the variant fertilized with 40 t / ha city sludge). Also, all experimental variants in the two years 2010-2011, the percentage of nitrogen do not show major differences compared to the control unfertilized (0.24% in
horizon Bt horizon I and 0.20 in the control variant to 0.26 % in the horizon I and 0.23% in the Bt horizon variant fertilized with 40 t/ha city sludge). Quantities of phosphorus (41ppm and 12ppm in the horizon I Bt horizon to version control from 44ppm to 15ppm horizon to horizon Bt I and the variant fertilized with 40 t / ha city sludge) and potassium (342ppm and 200ppm in the horizon I Bt horizon to version control from 342ppm to 207ppm horizon and the Bt horizon I the variant fertilized with 40 t / ha city sludge) in the soil increased slightly in variants with application of city sludge. Also the percentage of humus was very little influenced by sludge (4.33% in the horizon I and 3.64 in the control variant Bt horizon to horizon I 4.35% and 3.67% in the Bt horizon of the variant fertilized with 40 t/ha city sludge).

Therefore application of city sludge from wastewater treatment plant in Cluj-Napoca Tetarom III in different doses (20, 30, 40 and 60 t/ha sludge) did not cause significant changes in chemical characteristics (pH, CaCO3, total nitrogen, phosphorus, potassium, humus and volumetric weight) compared to the control soil fertilized or first experimental year, 2010 or 2011 in the second year of the application of city sludge.

6.3. THE INFLUENCE OF CITY SLUDGE APPLICATION UPON MICROBIOLOGICAL MARKERS

First measurement of soil respiration was performed in July (19.2 °C) of 2011, and after it, there were differences between version control and application of city sludge variants and manure. The biggest differences in terms of soil respiration were recorded in variants fertilized with 40, 60 t/ha city sludge and 20 t/ha manure that were noted in statistically very significant. These values are: the variant treated with 60 t/ha sludge, which determined a value of 3.85 g/m²/h (with a difference of 0.69 g/m²/h compared to the control), and variant treated with 40 t/ha city sludge with a value of 3.68 g/m²/h (with a difference of 0.52 g/m²/h compared to the control). At the opposite end variant treated with 30 t/ha city sludge with a value of 3.23 g/m²/h soil respiration (with a difference of 0.07 g/m²/h compared to the control). These issues lead us to affirm the fact that the higher dose of sludge is much higher soil respiration values are also higher.
The second measure soil respiration was performed in October (8.8 °C), and statistically speaking, in all experimental variants were recorded very significant values compared to the control unfertilized.

The highest values of soil respiration in October of 2011 occurred in the variant treated with 40 t/ha city sludge, with a value of 2.78 g/m²/h (with a difference of 0.66 g/m²/h compared to the control), and that the variant treated with 60 t/ha manure, with a value of 2.75 g/m²/h (with a difference of 0.63 compared to the control g/m²/h). The lowest value was observed in the variant treated with 60 t/ha city sludge g/m²/h 2.43 (with a difference of 0.31 g/m²/h compared to the control).

Fertilization with manure at 40 t/ha leads to a value of 2.66 g/m²/h soil respiration approximately equal to that of the fertilization with city sludge at a dose of 20 t/ha, which had a value of 2.61 g/m²/h. It was noted that in October of 2011, the greater the dose of city sludge, the soil respiration value increases, except for the variant treated with 60 t/ha sludge (soil respiration with a value of 2.43 g/m²/h). The same thing happens when manure.

Symbiotically fixed nitrogen values in variants treated with city sludge ranged from 122.09 to 131.35 kg/ha NFB and the variants fertilized with manure were from 117.02 kg/ha to 127.71 NFB kg/ha NFB.

The highest amount of biologically fixed nitrogen was in the variant treated with 40 t/ha sludge about 131.35 kg/ha (with a difference of 24.11 kg/ha compared to the control) was higher variant fertilized with the same amount manure that set 127.61 kg/ha nitrogen (with a difference of 20.47 kg / ha compared to the control) recorded a value of 3.64 kg / ha nitrogen for variant treated sludge, and the smallest amount of biologically fixed nitrogen was observed in unfertilized control variant, namely 109.41 ka/ha nitrogen.

It proves that the fertilization with city sludge increased doses there is no increased activity symbiotic nitrogen fixing bacteria to economically justify the application of these doses. Regarding fertilization with manure at 40 t/ha, this leads to an amount of biologically fixed nitrogen fertilization equal to 20 t/ha city sludge (both fixed amount of 127.71 kg / ha nitrogen ). On the contrary application of 60 t/ha manure leads to reducing the amount of symbiotically fixed nitrogen at 117, 02 kg / ha comparable unfertilized control variant of nitrogen fixing the amount of 107.24 t / ha. Fertilization
with manure increased dose not justified economically and prevent symbiotic nitrogen fixation.

Increasing the dose from 40 to 60 t/ha city sludge resulted in reduced NFB's because bacterial activity is inhibited by excess sludge.

In the second experimental year 2011, like 2010, is maintained superiority variant treated with 40 t/ha city sludge which symbiotically fixed nitrogen increases the value 163.49 ka/ha (showing a difference of 42.51 kg/ha compared to the control), superiority that manifests both the other variants tested and compared to the variant fertilized with the same amount of manure that is an amount of 159.46 oxide (a difference of 38.45 kg/ha compared to the control), and the smallest amount of biologically fixed nitrogen was obtained in version control, 120.98 kg/ha. From all statistically very significant variations were dashed.

In 2011, in terms of quantity NFB to obtain more significant differences between the variants treated with city sludge or manure, compared to the control than in 2010, the NFB small difference compared to the control was in version treated with 30 t/ha city sludge (28.28 kg/ha NFB), while the opposite is the variant treated with 40 t/ha city sludge that secures 42.51 kg/ha nitrogen.

Regarding fertilization with city sludge at 60 t/ha, which determines the amount of biologically fixed nitrogen to 152.54 kg/ha, amount approximately equal to the amount of symbiotically fixed nitrogen from application of the same dose of manure (153.74 kg/ha NFB).

There is otherwise a direct link between production growth through the application of sludge and NFB, as confirmed by our results showing an increase in NFB in all experimental variants.

Sludge application, so rich in N, P, K, and micronutrients increases the work efficiency fixing symbiotic nitrogen fixing bacteria and therefore the amount of biologically fixed nitrogen.

In our experiment we decided to study symbiotic nitrogen fixation efficiency in alfalfa in experimental field conditions in Bolduți.
This study involves the application of technology with culture for the species under study, monitoring fixing symbiotic efficiency indices namely nodule the plant number, size, shape and color nodule, plant layout.

The root system of alfalfa, which hosts mechanism of atmospheric nitrogen fixation, the intensity of this process, there are a number of morphological changes (size, shape and number pf nodule), physiological (water absorption capacity and mineral salts) and chemical (nitrogen content level). If alfalfa crop, an increase of nitrogen content and a slight increase in the number and size of nodule as a consequence of city sludge.

If alfalfa crop, an increase of nitrogen content and a slight increase in the number and size of nodule as a consequence of city sludge.

To reveal the morphological and tinctorial nodule native preparations were made and fixed. Smears were viewed and photographed in digital trinocular microscope. In preparations obtained directly from nodule appeared and bacillary forms. Literature indicates that these forms are inactive, so do not fix atmospheric nitrogen.

Based on the effects on plant nodule were classified as effective or ineffective. The Efficient large and forms the root tip. Nodule functional efficiency is a fundamental quality of symbiosis. Plant infection by a strain of *Rhizobium* causes inefficient depletion through intensive plant photosynthesis products.

Observations on morphological characters and tinctorial root nodule phenophase were performed both in the buds and the phenophase of flowering alfalfa.

The first part of vegetation, to form nodule both host plants and nodule bacteria need nitrogen. Biological nitrogen fixation for early start is necessary for the soil to find relatively small amount of nitrogen (15-20 kg N/ha). Too much nitrogen inhibit the nodule, nitrogenaza bacterial enzyme.

The examination of morphological and tinctorial characters of nodule the phenophase of buds was observed that the number of nodule is lower during this period, ranging between 12 and 20 nodule the plant. These bacteria have a oval-elongated and sometimes branched in two or more directions.

Nodule dimensions have values between 1.2 to 2.5 µm presents that one white white rosacea.
The examination of morphological and tinctorial characters of nodule the flowering phenophase was an increased intensity of nodules number from 39 to 64 per plant compared nodule number of phenophase buds. These bacteria are formed in large numbers and are effective in the active period of alfalfa and active thereafter gradually reduced nodule number. Nodule of alfalfa in this phenophase have shaped stick, giving a length between 13.5 to 19.1 µm in 2010 and from 13.4 to 18.9 µm in 2011.

Active nodule fixation phase has a red color, pink, red conferred by leghemoglobin. After the fixing activities, training phenophase asemintei, nodules color is green, and they become inactive.

Both in 2010 and in 2011 the study was conducted at nodules phenophase of flowering alfalfa in knowing that symbiotic fixation has the highest flowering intensity pulses.

In May of 2010 were analyzed first alfalfa plants, highlighting the presence of nodules at the roots. Genus *Rhizobium* bacteria invade the roots of leguminous plants and induces the nodule. The largest number of nodule was recorded at variant treated with 40 t/ha city sludge with a total of 46 nodules per plant (with a difference of 7 nodule compared to control), and at the opposite end variant treated with 60 t/ha manure which recorded a 41-nodules per plant compared to 39 in the unfertilized control variant.

Fertilization with high doses of sludge (60 t/ha) inhibit these nodule. Fertilization with manure in the first experimental year the plant does not affect the number of nodule applied only at the highest dose (60 t/ha) being able to see an increase in this number. Instead dose of 20 t/ha had only a significant increase in the number of nodule compared to the control (42 to 39).

The second analysis of nodule bacteria was conducted in late May of 2011. Just as in the first year, the variant treated with 40 t/ha city sludge where there is a number of 64 nodule per plant (compared to 53 controls and 60 nodules the variant fertilized with the same dose of manure).

The lowest number of nodule per plant was observed in the variant fertilized with 60 t/ha city sludge which recorded a 53 nodules (not registered is no difference compared to control).
The number of nodules in experimental year 2011 (values between 53-64 nodule per plant) is higher than in 2010 (values between 39-46 per plant), which leads us to say that the effect of city sludge on nodule bacteria is felt much better in the second year of application, when it comes to the bacteria of the genus *Rhizobium* a significant amount of nutrients designed to support the work of fixing symbiotic.

In phenophase of flowering, while nodules counting was performed and their microscopic measurement. Smears were viewed and photographed in digital trinocular microscope.

In 2010 the largest nodule sizes were observed in the variant treated with 40 t/ha city sludge (19.1 m) to 13.5 $\mu$m in untreated control variant. Smallest were found in variant treated with 60 t/ha manure (13.9 m) with a difference of 0.4 $\mu$m compared to the control.

In statistical terms in all experimental variants were scored very significant.

Determination of nodule in the second experimental year 2011 varied as in 2010 and the largest size of nodules were observed in the variant treated with 40 t/ha city sludge (18.9 m) with a difference of 5 5 mm compared to the control (13.4 m). Smallest were found in variant treated with 60 t/ha manure (14.2 m) with a difference of 0.8 $\mu$m compared to the control.

**CHAPTER VII**

**PARTIAL CONCLUSIONS**

1. Recovery city sludge in agriculture is an effective method to replace mineral fertilization, ensuring at the same time, reintegration circuit sludge material in nature.
2. Data on microbiological activity in the experimental field from Boldut, indicators related soil microbiological and those of alfalfa productivity under different conditions show that replacement fertility with manure sludge arising from wastewater treatment plants Town is a solution worthy of consideration can be justified and economically.
3. Using sludge from the treatment plant in Cluj-Napoca Tetarom III is conditional upon its content of heavy metals in crops is compulsory analyze chemical traits, especially the heavy metal content.
4. Agricultural species, application of city sludge is provided that the tolerance to be above par. In alfalfa grown in the experimental field from Boldut, Cluj County, the tolerance values were calculated species that express tolerance to moderate doses of sludge (40 t/ha).

5. Sludge fertilization in moderate doses (up to 40 t/ha) did not cause changes in the characteristics, soil chemical toxicity in the experimental field from Boldut, Cluj County.

6. Intensity of soil biological activity expressed by values of soil respiration is positively influenced by fertilization with city sludge derived from wastewater treatment plants.

7. Number and size of nodule are significantly influenced by fertilization with city sludge. It notes, however, an increasing number of nodules in the second year after fertilization mud correlated with increased symbiotic nitrogen fixed (NFB).

8. Increase the amount of NFB hub city sludge fertilization dose increased (122.09 to 131.35 t/ha NFB), which correlates with production increases obtained.

9. In the second experimental year the NFB has higher values sludge fertilization and fertilization with manure (149.35 to 163.59 t/ha respectively NFB 151.56 to 159.53 t/ha NFB) in moderate doses, given the provision of micro amounts of micro and macro fixing symbiotic useful.

10. DM in alfalfa production is influenced very significantly by fertilization with city sludge, resulting in production of: 8.45 to 9.16 t/ha DM in the first experimental year and 10.55 respectively to 11.64 t/ha DM in the second experimental year. The highest production increase is obtained in the second year of sludge application in the variant fertilized with 40 t/ha citysludge (11.64 t/ha DM).

11. Fertilization with manure resulted in achieving production increases very significant statistically (8.06 to 8.88 t/ha DM in the first experimental year respectively 10.71 to 11.33 t/ha DM in the second experimental year). The highest increase production to fertilization with manure was obtained in the variant fertilized with 40 t/ha (11.33 t/ha DM).

12. Alfalfa forage quality is not affected by fertilization with city sludge from WWTP Tetarom III or in the first year or the second year of its application.
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