

## ASPECTS CONCERNING GROWING FLORICULTURAL SPECIES *Hydrangea macrophylla* ON THE SUBSTRATE WITH COMPOST AND MICROORGANISMS PRESENT IN THESE SUBSTRATES

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**Abstract.** Organic fertilization with compost derived from municipal treatment plants represent at least up to now disputed fertilization, its effects in horticulture are contradictory, due to presence of heavy metals and possible pathogens. The main objective of this paper was to exploit and identify microbiological risks associated with the use of compost made from municipal household waste mixed with vegetable waste by applying it as rooting substrate for floricultural species *Hydrangea macrophylla*, assessing the microorganisms present in these substrates. This paper presents the results of the microbial load determination in four variants (V2-V5) in comparison with the control variant (V1).

**Keywords:** fermented sludge, composting, *Hydrangea macrophylla*, pathogenic microorganisms.

**Rezumat. Aspecte privind creșterea speciei floricole *Hydrangea macrophylla* pe substrat cu compost și microorganismele prezente în aceste substraturi nutritive.** Fertilizarea organică cu compost provenit din stațiile de epurare municipale reprezintă cel puțin până în prezent o fertilizare controversată, efectele sale în sectorul horticol fiind contradictorii, datorită prezenței metalelor grele și posibilelor microorganisme patogene. Obiectivul principal al lucrării de față a fost acela de a valorifica și de a identifica riscurile microbiologice asociate utilizării compostului obținut din deșeuri menajere municipale în amestec cu deșeuri vegetale prin aplicarea acestuia ca substrat de înrădăcinare pentru specia floricolă *Hydrangea macrophylla*, evaluând microorganismele prezente în aceste substraturi. Lucrarea prezintă rezultatele determinărilor microbiologice din cele patru variante experimentale (V2-V5) în comparație cu proba martor.

**Cuvinte cheie:** nămol fermentat, compostare, *Hydrangea macrophylla*, microorganisme patogene.

### INTRODUCTION

“Sewage sludge” or “biosolids” is the insoluble residue produced during wastewater treatment and subsequent sludge stabilization procedures such as aerobic or anaerobic digestion (ARTHURSON, 2008). Composting is the process by which organic materials such as sewage sludge is decomposed and stabilized under aerobic conditions, which support the development of thermophilic microorganisms (HIGHLAND *et al.*, 2004; HERSELMAN *et al.*, 2008). According to BERNAL *et al.* (1998b), MC CLINKTOCK (2004) compost maturity and stability imply lack of phytotoxic compounds and pathogens. Microbial activity is widely accepted as the most reliable indicator of compost stability and several studies have attempted to correlate the physical and chemical parameters with breathing. Pathogens survive in compost a limited time depending on microbial species and conditions it offers. This leads to the need to establish to what extent and under what conditions the compost can be responsible for transmitting the disease. Studies confirm that it can be contaminated without carrier of pathogens, but their occurrence can ever become possible (JIANLONG & JIAZHUO, 2007). In this case is used the term of indicator germs which may indicate whether the product studied is contaminated and therefore is able to become a broadcaster of pathogens. Similar experiments were performed by MARK *et al.*, 2004 by investigation plants test *Antirrhinum majus* “Rocket White”, *Viola × wittrockiana* “Crown Azure”, Oriental Hybrid *Lilium* “Siberia”, and *Chrysanthemum × grandiflorum* “Yellow Kodiak”.

PRASAD & FREITAS (2003) mentions that *Hydrangea* is part of plants used for laboratory experiments, the results serve to advance the knowledge of metal bioremediation and environmental monitoring as *Hydrangea* is a strong aluminum hiperaccumulator. WU *et al.*, 1995, studied the effects of regenerated wastewater irrigation on growth and ions uptake in many plants, inclusive in *Hydrangea macrophylla*.

### MATERIAL AND METHODS

Fermented sludge analyzed in accordance with Government Decision 708/2005 technology in the laboratory of the Institute INCD-ECOIND Bucharest was mixed with biodegradable vegetable wastes, the proportion was 70% sludge from sewage treatment plant Pitesti and 30% of the vegetable residues (leaves, grass, straw), a total of 1 m<sup>3</sup> of mixture, obtaining the final product compost at Albota Experimental Research Station. The research program used fermented sludge collected from Wastewater Treatment Plant Pitesti, compost from Albota Experimental Research Station, as nutrient substrates for floricultural species *Hydrangea macrophylla*. Their vegetation grown process was studied in pots in the greenhouse environmental conditions at Craiova Botanical Garden belonging to the Discipline of Floriculture - Faculty of Agriculture and Horticulture from Craiova.

Has been developed a mono-factorial experience type, following the present microbial community in the substrate for *Hydrangea macrophylla* – 50 seedlings and the cuttings percentage (during the period December-March) on five experimental variants:

- V1 - peat: compost: perlite (1: 0: 1)  
 V2 - peat: compost: perlite (0: 5: 1.5)  
 V3 - peat: compost: perlite (2.5: 2.5: 1.5)  
 V4 - peat: compost: perlite (1: 4: 1.5)  
 V5 - peat: compost: perlite (3: 2: 1.5).

It is known that the waste water, waste sludge and compost have a very rich indigenous bacterial biocoenosis which is developing at relatively low temperatures, so the research studies involves the total mesophilic germs and total number of germs (TNG) (JIANLONG & JIAZHUO, 2007). All known characteristics are taken into consideration for the classification of microorganisms, but certain features are selected and used for identification purposes.

Usually that involves some simple tests to identify primary such as morphology (usually by Gram stain), growth in the presence or absence of O<sub>2</sub>, growth on various types of culture media, catalase and oxidase tests. Using these few simple tests is usually possible to place provisional microorganism, in one of the main groups. The compost well homogenized sample was weighed at 100 g sediment and placed in a sterile bottle containing glass beads. Was added 1000 cm<sup>3</sup> sterile distilled water and shaken through magnetic stirrer for 30 minutes to make suspension of germs, then leave to stand for 5 minutes settling coarse particles (dilution 1/10). From the obtained supernatant were prepared decimal dilutions in sterile buffered water. Frequently made to 1/100.000 dilutions to obtain a density suitable microbial identifications. Quantitative determination of total and faecal coliform is required in order to evaluate quality in terms of bacteriological using multiple tube method, which provides assessment data the most likely germ Most Probable Number (MPN) (SIDHU *et al.*, 1999).

Determining Most Probable Number of coliform per gram is done using probability tables McCrady. Various combinations of positive and negative results allow to estimate the probable number of total coliform (TC), faecal coliform (FC), total streptococci (TS) and faecal streptococci (FS).

The following tests were used in the experiment:

- To identify group D streptococci - Strep Pastorex agglutination rapid tests for streptococci of Lancefield classification.
- To identify *Staphylococcus aureus* - latex agglutination rapid tests for simultaneous detection of antigen affinity fibrinogen (clumping factor), protein A and capsular polysaccharides of *Staphylococcus aureus*: (Bio-Rad Pastorex ® Staph Plus kit).
- For yeasts and moulds - Fungitest™ studying test fungi growth in the presence of 6 antifungal, each in two different concentrations. Fungal growth is based on the colour change from blue to pink. When fungal growth is inhibited, the wells are keeping their blue colour.

## RESULTS AND DISCUSSIONS

Microbiological test results are presented in table 1 and figure 1.

Table 1. Microbial load of the experimental variants of compost-perlite mix-peat substrate MPN/g. / Tabel 1. Încărcătura microbiană din variantele experimentale ce conțin ca substrat amestecul de compost-perlit-turbă MPN/g.

Experimental variants	Total coliform/dm <sup>3</sup>	Faecal coliform/dm <sup>3</sup>	<i>Escherichia coli</i> / dm <sup>3</sup>	Total streptococcus/dm <sup>3</sup>	Faecal streptococcus/dm <sup>3</sup>	Total aerobic bacteria (UFC) / cm <sup>3</sup>	Observations
V1 control variant	13 · 10 <sup>2</sup>	< 20	< 20	< 20	< 20	6 · 10 <sup>3</sup>	<i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus</i> , yeasts and molds = absent
V2	2 · 10 <sup>2</sup>	< 20	< 20	< 20	< 20	7 · 10 <sup>3</sup>	<i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus</i> , yeasts and molds = absent
V3	5 · 10 <sup>4</sup>	< 20	< 20	2 · 10 <sup>4</sup>	2 · 10 <sup>2</sup>	9 · 10 <sup>4</sup>	<i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus</i> , yeasts and molds = absent
V4	2 · 10 <sup>4</sup>	< 20	< 20	3 · 10 <sup>4</sup>	3 · 10 <sup>2</sup>	7 · 10 <sup>6</sup>	<i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus</i> , yeasts and molds = absent
V5	5 · 10 <sup>3</sup>	< 20	< 20	3 · 10 <sup>3</sup>	8 · 10 <sup>2</sup>	11 · 10 <sup>4</sup>	<i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus</i> , yeasts and molds = absent

Concerning the microbial load (TNG) in compost-perlite mix-peat substrate it was observed that samples analyzed microbiologically (MPN/g < 2 x 10<sup>6</sup>/g colony forming units), quality qualifies for the U.S. Environmental Protection Agency for compost that can be used in agriculture.

The results of the performed microbiological analyzes showed that although compost presented MPN values of germs/g of order 10<sup>2</sup> to 10<sup>4</sup>; it could be considered that it has no risk of contamination by its application in agriculture domain. It was also observed the absence of pathogens such as *Shigella*, *Salmonella*, *Staphylococcus aureus* species.

Analyzing the microbial load through the types of germs, for the five studied variants was observed the following aspects:

- For total coliform (TC) - MPN/g has the highest value in V3 variant and the lowest in variant V2;
- For faecal coliform (FC) - MPN/g values were identified in <20 in all experimental variants;
- *Escherichia coli* - MPN/g had identified values of <20 in all experimental variants;
- Total streptococci (TS), faecal streptococci (FS) had slightly elevated values MPN/g for variants V3, V4 and V5, but low risk of contamination, while the variants V1 and V2 recorded lowest values.

The highest total aerobic bacteria number was recorded in V4 variant and the lowest one in variant V1. Comparing the total microbial load of the analyzed compost - perlite mix-peat substrate in all five variants it has been observed that microbial load in V4 and V5 have the highest and lowest version in V2 (version V1 as control). V2 could be considered optimal way of nutrient substrate for floriculture area.

- Faecal coliform (FC) MPN/g values were identified in <20 in all experimental variants;
- *Escherichia coli*, MPN/g values was identified in <20 in all experimental variants.

La nivel global, rezultatele din tabelul nr.1 au arătat un grad mic de contaminare a compostului cu microorganisme fecale și ca atare valorificarea sa în agricultură va avea efecte benefice.

The analyzed variants were used as substrate for rooting and growing floricultural species *Hydrangea* sp. obtaining the following catch percent of cuttings (Fig. 1).

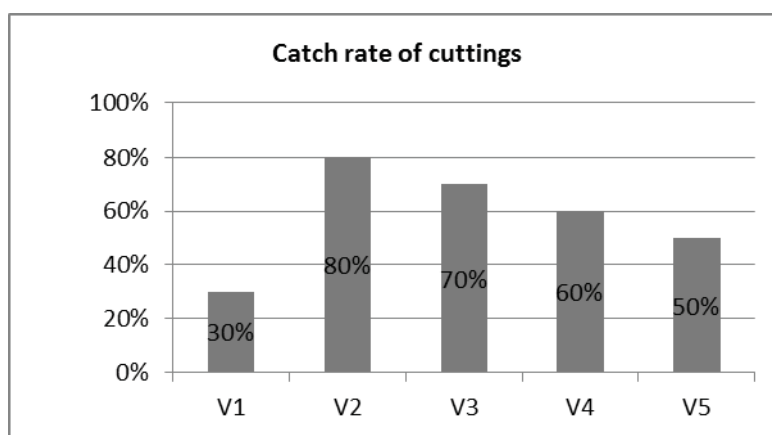


Figure 1. Catch percentage of cuttings. / Figura 1. Procentul de prindere al butășilor.

It could be appreciated that the low number of pathogens in V2 variant could represent an additional factor of stimulating the caulogenesis process.

## CONCLUSIONS

Based on the obtained results it can be concluded that:

- The process of composting, although the fact that its sterilizing effect is not total for all potentially pathogenic bacteria, may still be considered as one of the most effective methods for decontamination of waste products for soil fertilization.

- Our researching results showed a low degree of contamination with faecal microorganisms in compost-perlite mix-peat substrate studied variants and it could be recommended for capitalization in floriculture.

- Applying the compost obtained from fermented sludge mixed with residues plants as rooting substrate for floricultural species *Hydrangea* sp., it was observed that variant V2 presented the highest percentage of attachment cuttings.

- Analysing the microbial load as pathogen incidence demonstrates that compost can be applied without risk of contamination of soil and for manipulating people, with respect to the environment.

- Effects of using compost in the horticultural sector are diverse and not yet fully elucidated, despite the theoretical premises, in practice it fails to reduce the risk due pathogens present. Consequently, at least for now, organic fertilizer and compost derived from municipal sludge treatment plants will not only show in horticulture as a preventive measure and in accordance with the law.

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