A SNAPSHOT OF MESO- AND MACROORGANISMS IN COMPOSTING HOP WASTE BIOMASS

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Abstract
This study offers a snapshot of meso- and macroorganisms inside the composting pile of hop biomass after harvest (stems and leaves of hop plants) after five months of on-site composting. Three composting piles (three treatments) were prepared right after the harvest in September 2021. Composting technology was performed the same way for all of them (turning the pile according to the daily measurements of the temperature, after turning the pile is formed back into a trapezoidal shape each time, covering with a permeable membrane after the thermophilic phase), differences among piles were in the initial size of the plant particles, in composting additives added at the piles preparation (biochar, effective microorganisms, no additive) and in the number of pile turnings. In January 2022, eight different meso- and macrofauna species were detected. Phylum Arthropoda species were the most dominant in all piles. The mesofauna was dominated by Springtails (Collembola) in size between 100 µm and 2 mm and Mites. The macrofauna was dominated by Springtails (Collembola) in size 2 mm to 20 mm wide, Spiders, Centipedes, Soldier flies, and larve of Fungus Gnats. In the pile with biochar there was lower diversity than in the composting pile with effective microorganisms and in the one without additive, probably because temperatures in the thermophilic phase in this pile were reaching above 65°C. There were just springtails, mites and larvae present in that pile. The highest diversity was in the composting pile with small parts of hop stems and with effective microorganisms added at the beginning.
Keywords: *Humulus lupulus* L., hop waste biomass, composting biomass, compost fauna, Arthropoda, biodiversity, LIFE project, BioTHOP

MEZO- IN MAKROORGANIZMI V KOMPOSTIRAJOČI HMELJEVINI

Izvleček
Študija podaja vpogled na mezo- in makrofavno v kompostirajoči hmeljevini (stebla

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in listi hmelja - biomasa, ki ostane po obiranju hmelja) po petih mesecih kompostiranja. V septembru 2021, dan po obiranju hmelja, smo pripravili tri kompostne kupe (obravnavanja). Pri vseh je bila tehnologija kompostiranja izvedena enako (obračanje kupa glede na dnevne meritve temperatur v kupu, kup vsakič po obračanju oblikovan nazaj v trapezoidno obliko, pokrivanje s permeabilno membrano po termofilni fazi), razlike med njimi pa so bile v začetni velikosti delcev biomase, v dodatkih za kompostiranje, ki so bili dodani ob pripravi kupov (biooglje, efektivni mikroorganizmi, brez dodatka) in v številu obračanja kupa. V januarju 2022 smo odkrili osem različnih skupin mezo- in makrofavne, med njimi so v vseh kupih prevladovali predstavniki debla členonožcev. Najbolj pogosti predstavniki mezofavne so bili skakači velikosti 100 µm do 2 mm in pršice, makrofavne pa skakači velikosti 2 mm do 20 mm, pajki, stonoge, mrtvaške mušice ali žalovalke in ličinke muh. V kupu z biooglјem je bila pestrost manjša kot v kupu z efektivnimi mikroorganizmi in v kupu brez dodatkov, verjetno zato, ker so temperature v termofilni fazi v tem kupu večkrat presegle 65ºC. V tem kupu so bili le skakači, pršice in ličinke žuželk. Največja pestrost mezo- in makrofavne je bila v kompostnem kupu z majhnimi začetnimi delci stebel in dodanimi efektivnimi mikroorganizmi.
Ključne besede: *Humulus lupulus* L., hmeljevina, rastlinska biomasa, kompostiranje, kompost, favna komposta, členonožci, biodiverziteta, LIFE projekt, BioTHOP

1 INTRODUCTION

Composting is a process in which the organic matter gets degraded by microbes under aerobic conditions to obtain a stable material that can be used as organic fertilizer (Bustamante et al. 2010). The trend towards more efficient methods of compost production and handling requires a complete understanding of the process, the materials involved, and the physical parameters of the materials such as moisture content, bulk density, and various mechanical properties (Agnew, 2003). However, to obtain a high-quality compost it is necessary to understand the process involved as well as to evaluate the most suitable performance conditions for certain biomass (Khater, 2015).

Composting hop waste biomass (stems and leaves of hop plants that stay next to the harvest hall after the cones harvest) on hop farms and use the compost for fertilization is one of the ways to close the nutrient cycle in hop farms, as well as introduce circular economy. Within the European project LIFE BioTHOP, biodegradable and compostable twine made from polylactic acid (PLA) was being developed as a replacement for plastic twine, which serves for climbing hop plants support during growth nowadays. This makes post-harvest biomass processing easier, as the whole mixture of biomass and BioTHOP twine degrades together at proper composting conditions and there are no artificial leftovers in the final product. The guidelines for proper on-farming composting have to be defined to get good and safe compost. Luskar et al. (2021) found out that under controlled on-farm conditions composting hop waste biomass intertwined with BioTHOP PLA twine exceeded the minimum limit temperature (55°C) for PLA degradation and biomass hygeinization. The final composts in that reaserch exceeded the average concentration of macronutrients (C, N, P, K) for hop biomass compost and compost's water extracts had a good effect on cress seed germination, meaning that composts were not toxic, contrary they had positive effect. Luskar and Čeh (2021) reported, that hop biomass composting approach has a significant impact on compost microbiological properties.

Compost is normally populated by three general categories of microorganisms: bacteria, actinomycetes and fungi. It is primarily the bacteria, and specifically the thermophilic bacteria (Sánchez et al., 2017). While processes of nutrient cycling are governed directly by microbes, such as bacteria and fungi, they are also affected by soil animals that live alongside them. Soil fauna affect decomposition processes both directly, through fragmentation and comminution of litter material, and indirectly by altering microbial function through grazing of the soil microbial biomass and through excretion of nutrient rich wastes. The movement of animals through soil influences the dispersal of microbial propagules attached to the animal body surfaces or transiting through their guts (Cole et al., 2006). Invertebrates co-exist with the microbes and are essential to a healthy compost pile. One classification into three groups (micro-, meso- and macroorganisms) derives from their size and the way they interact with their habitats (Anderson, 1988).

Microfauna are protozoa and invertebrates of less than 100 µm, mostly nematodes. The participants of diverse group of mesofauna, invertebrates, are of sufficient size to overcome the surface tension of water on soil particles but are not large enough to disrupt the soil structure in their movement through soil pores (body width between 100 µm and 2 mm). They include Acari (mites), Collembola (springtails), enchytraeid worms, small Diplopoda (millipedes), and many small larval and adult insects. Acari, and Collembola are being by far the most abundant and diverse (Culliney, 2013). Studies of the mesofauna have concentrated on springtails and mites. This group consists principally of species of the acarine taxa Oribatida, Prostigmata, and Mesostigmata, and the Collembola. Large numbers of microarthropods are found in most soils in a variety of environments from equatorial to polar regions and from temperate and tropical forests and grasslands to hot and cold deserts. As part of the mesofauna, the microarthropods comprise the important middle links of soil food webs, serving, in their role as both predator and prey, to channel energy from the soil microflora and microfauna to the macrofauna on higher trophic levels (Culliney, 2013).

Macrofauna consists of species large enough to disrupt the soil by their burrowing and feeding (2 mm to 20 mm wide). The most important taxa are Isopoda (woodlice), larger Diplopoda, earthworms, Isoptera (termites), Coleoptera (beetles), Diptera (flies), ants, and molluscs (snails, slugs). They are more active in the later stages of the composting process, when temperatures have dropped but decomposition is not complete (Culliney, 2013). Arthropods function are on two of the three broad levels of organization of the soil food web (Lavelle, 1995): they are “litter transformers” or “ecosystem engineers.” Litter transformers, of which the microarthropods comprise a large part, fragment, or comminute, and humidify ingested plant debris, improving its quality as a substrate for microbial decomposition and fostering the growth and dispersal of microbial populations. Ecosystem engineers are those organisms that physically modify the habitat, directly or indirectly regulating the availability of resources to other species. In the soil, this entails altering soil structure, mineral and organic matter composition, and hydrology (Culliney, 2013).

These organisms play a very important role in ecosystems. A food web is a complex interaction of food chains in a biological community. In order to understand the decomposition process within an ecosystem, it is essential to assess the populations of these groups. The microbial mineralization of nutrients may be stimulated by arthropod grazing. Several studies have demonstrated that grazing by Collembola has a strong stimulatory effect on fungal growth and respiration (Filser, 2002; Culliney, 2013).

The microbial world of final compost from hop biomass (no other biomass added) in one of the early investigations by Luskar and Čeh (2021) was dominant by bacteria and it lacked microbial diversity, which is an important property of quality compost. Fast changing conditions in compost demand fast adaptation of microbes that can only be tackled by diversity. The most biodiverse compost was the one with biochar added at the beginning, followed by compost with no additive and finally by the compost with effective microorganisms added. Compost with no additive and compost with biochar had many amoebae, while compost with effective microorganisms lack these organisms. Bacteria are preyed upon by protozoa and nematodes, while fungi are preyed upon amoebae, nematodes, and micro-arthropods. Part of mycelium was found only in compost where biochar was added and, in the compost, with no additive. In general, all composts lacked diversity, which is main property of quality compost. Due to their fast reproduction, the number does not play such important role as their diversity.

The goal of composting would be compost, rich in nutrients, safe and also rich in microorganisms and in fauna. The aim of this study was to take a snapshot of diversity of mesofauna and macrofauna in composting hop biomass (stems and leaves of hop plants after hop cones harvest, no other biomass added) in winter - after four months of on-site composting (in January 2022).

2 MATERIALS AND METHODS

2.1 Experiment layout and weather conditions

Experiment was conducted in September 2021 in Lower Savinja Valley, Slovenia (Žalec). Each of three composting piles was prepared right after hop cones harvest in Sept. 2021 in a trapezoidal shape with a height of 2 m from approximately 15 tonnes of biomass. Differences among piles were in the initial size of the biomass particles, in composting additives which were added at the beginning of composting (biochar, effective microorganisms - EM™, no additive) and in the number of pile turnings, which were done in correspondence to the regular temperature measurements (Table 1). Biochar named Biočar from Slovenian company C-produkt d.o.o. (activated carbon prepared from soft wood varieties) and EM™ from company named Multikraft is a mixture of bran mixed with molasses (sugar and water), enriched with beneficial microorganisms (lactic acid bacteria, yeasts, photosynthetic organisms, enzymatically active fungi - over 80 different species of aerobic and anaerobic microorganisms; Multikraft, 2022) were used.

Table 1: *Composting pile parameters*

Composting pile	Initial size of hop stems (cm)	Additive, mixed in at the conduction of the pile	Pile turning	Duration of thermophilic phase* (days))	Average T in thermophilic phase* (°C)	Max measured T (°C)
A	2-5	Biochar (3,3 kg/t)	7 times	85	74,4	79,2
B	1-5	Effective microorganisms (EM™) (2 L/t)	4 times	52	71,8	76,1
C	2-10	No additive	4 times	29	57,5	68,9

* Temperatures in the pile above 50 °C.

Regular temperature measurements were performed in the first three months by inserting the thermometer 50 and 100 cm deep into the pile. Piles were turned / mixed when temperature was above 65 °C. The temperature in piles was measured by a modified compost digital thermometer probe. Because of different properties T was raising differently and that is also why the number of turnings, present in Table 1, is different related to the treatment. At the end of November 2021, we covered the piles with semipermeable cover until April 2022. Precipitation is not expected to interfere with composting process, because in the thermophilic phase, temperature and humidity inside the pile are key to successful composting, while most of the rain ran off the surface of a properly constructed pile and the pile was drying each time we turned/mixed it.

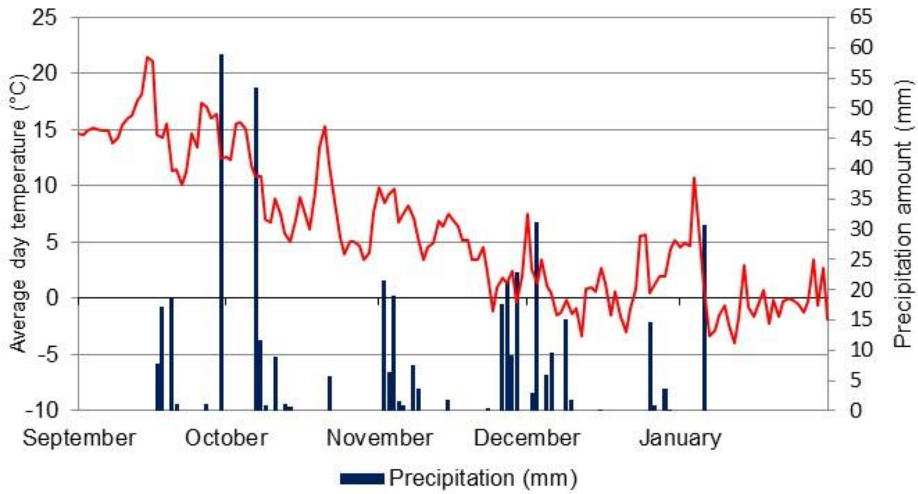


Figure 1: *Precipitation amount and average daily temperatures in the first four months of composting (from september 2021 to February 2022) (ARSO, 2022)*

2.2 Sampling and classification of fauna

For the present study, we made sampling of composting biomass twice in January 2022 at all three composting piles. Samples were handpicked randomly from two different levels in the piles (50 cm and 100 cm deep) and at different piles sides (north, south, east, west). Appr. 0.5 kg of biomass was collected at each pile this way for the study. A visual evaluation of the biomass was made at sampling as well, including smell, colour, estimation of biomass degradation, moisture content and PLA twine degradation observation. The mechanical method including hand sorting was used along with direct stereomicroscope observations for separation of insect fauna on petri dish. The collected biomass sample from each pile was well mixed and put on a tray (d= 33 cm). The biomass was split into four parts. From each quarter, we selected samples of about 2 grams biomass on 4 different places (marked with red circles on the Figure 1), shown on Figure 2. Sample of each quarter was putted on petri dish (d=7cm) and viewed under a Stereomicroscope. The results of observations were then summed at the end (8 g sample from 1 compost pile).

The present organisms were further identified and classified using identification keys. The references and keys used for the study were: *Živali naših tal* (The Animals of our Soil) by N. Mršič and *A general textbook of Entomology* by A.D. Imms (tenth edition 1977). Along with these two major references, some internet references (https://www.gbif.org/) were also used for the identification purpose. At the end, the results of observations were summed (8 g sample from 1 compost pile).

3 RESULTS AND DISCUSSION

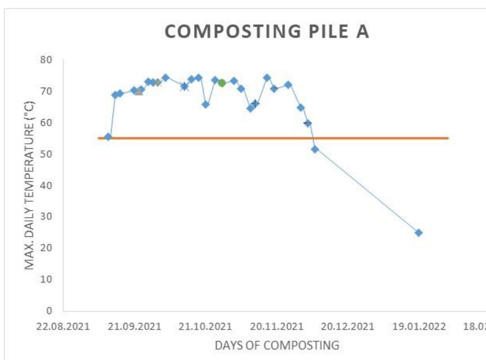
3.1 Temperature in the piles and visual evaluation

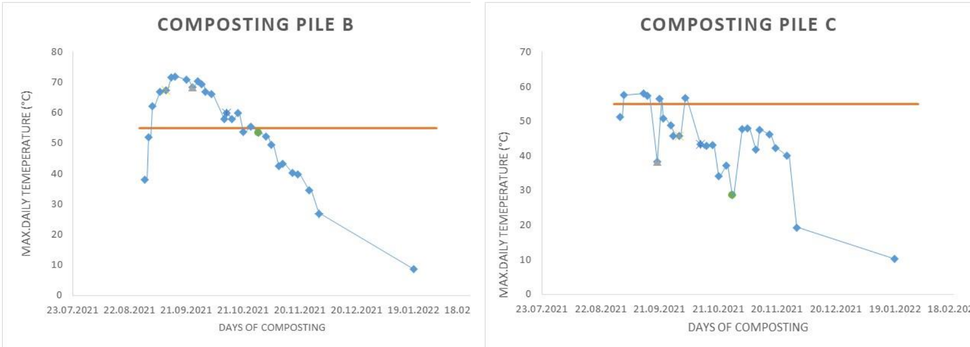
The thermophilic phase is not only important for elimination of plant pathogens and inactivation of weed seeds (Bollen and Volker, 1996) but also for PLA hydrolysis to lactic acid (Drumright et al., 2000; Garlotta, 2002; Kawai, 2010). However, too high temperatures on the other side can negatively affect compost parameters, including microorganisms. Thermophilic conditions were established one day after pile conduction already; the temperature increased up to 70 °C in the composting piles A and B (both with additives). The longest period of the thermophilic phase (temperatures above 75 °C) was detected in the pile A (Table 1). The temperatures reached in this pile were relatively high. Optimal temperatures in the thermophilic phase range between 45-65 °C (Amlinger, 2009).

In January 2022, in the time of taking samples, the temperature in composting piles B and C was app. 10 °C, while in the composting pile A was 45 °C and 12°C in the depth of 100 cm and 50 cm respectively. Material in composting piles with larger input particle sizes (A and C) was less degraded than material in the composting pile with smaller input particle size (B) at visual observation (Figures 4). BioTHOP PLA twine was still visible and poorly degraded (marked with blue elipse on the Figure 5), while in the composting pile with smaller particle input size and effective organisms added (B) there were only some remnants of the twine.

Composting piles B and C had an earthy smell, while sample from the pile A had a non-earthy, but not unpleasant smell, and it was darker.

Figure 2: Sampling method.





Figures 3: *Maximum daily temperature in the core of the composting pile, during first four months of composting in composting pile A (up right, composting pile B (down left) and composting pile C (down righ). Horizontal line is hygienisation treshold.*

Visual observation showed that the most optimally degraded compost was composting pile B. Also, Luskar et all. (2021) stated that the most suitable particle size for composting hop biomass is 5 cm or less. All compost had suitable temperatures to satisfy criteria for hygienisation and PLA hydrolysis to lactic acid.

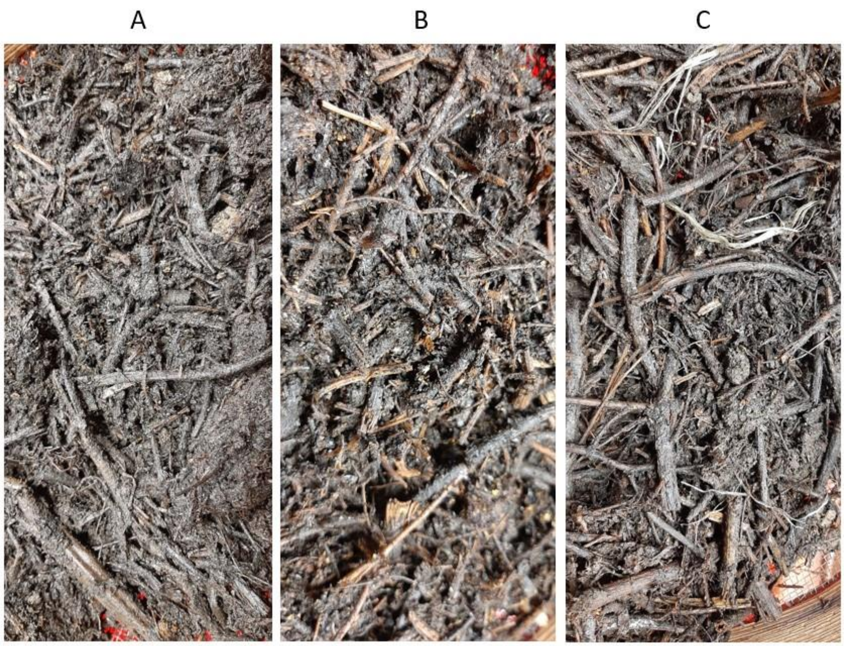


Figure 4: *Composting biomass samples – closer look after 5 months of composting related to the composting pile (A, B and C)*

Figure 5: *Composting biomass samples after 5 months of composting in piles A, B and C. The presence of the BioTHOP PLA twine is marked with ellipse.*

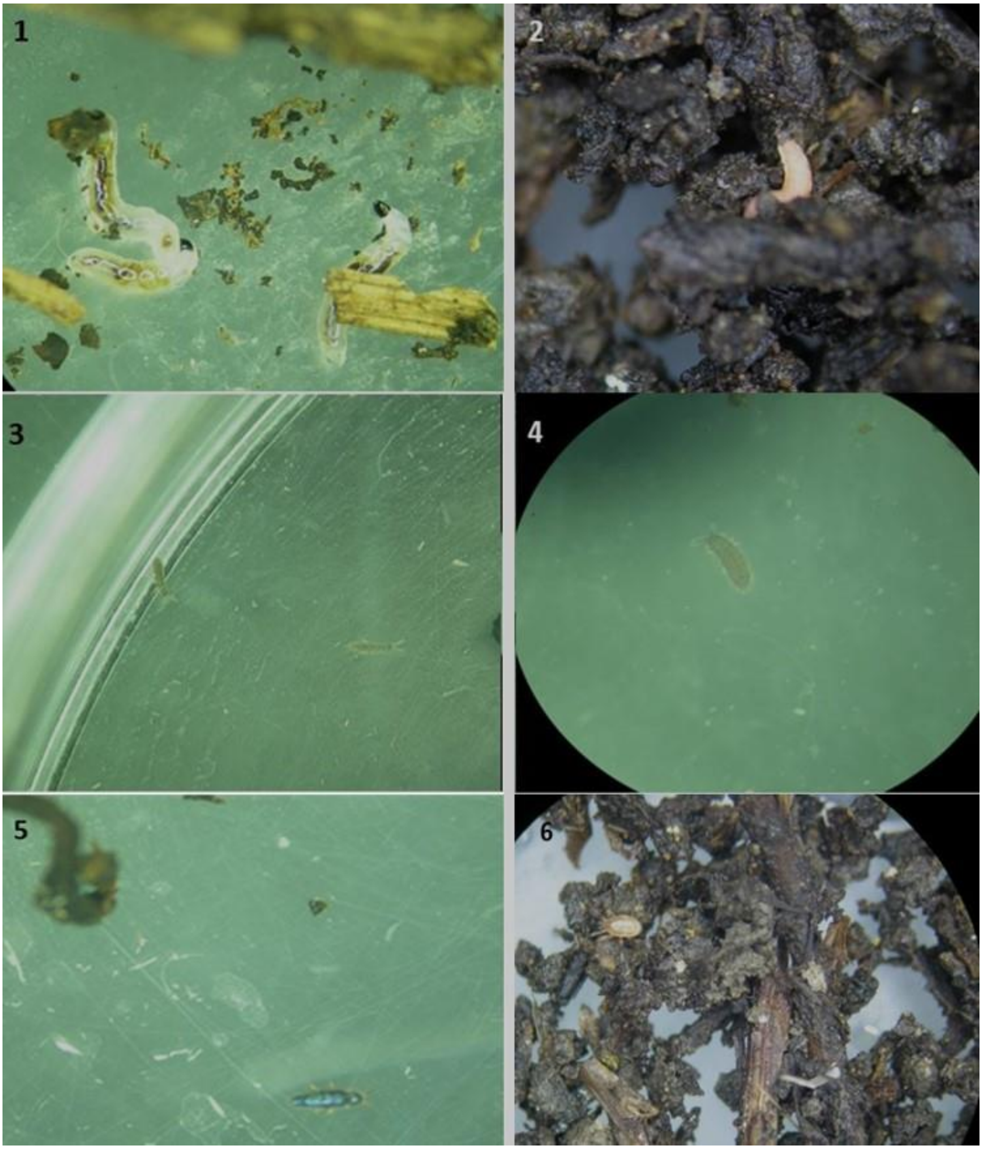
3.2 Identification of fauna

Table 2 presents results of detected mesofauna and macrofauna in the samples.

Table 2: *Organisms visually detected in the investigated composting piles in 8 g composting biomass samples*.

	Type	Composting pile A	Composting pile B	Composting pile C
Springtails	Mesofauna	More than 10 and different colours		
Mites	Mesofauna	More than 10	More than 10	More than 10
Centipedes	Macrofauna	Not detected	2	Not detected
Spiders	Macrofauna	Not detected	1	1
Soldier Fly	Macrofauna	Not detected	1	1
Rove Beetle	Macrofauna	Not detected	1	Not detected
Larve of Fungus Gnats	Macrofauna	2-5	2-5	2-5
Diptera Larva	Macrofauna	Not detected	1	Not detected

Figure 6 presents the detected organisms visually under stereomacroscope. In the investigated biomass samples, eight different fauna species were found. Phylum Arthropoda was dominant, among them phylum, class Insecta and class Arachnida.



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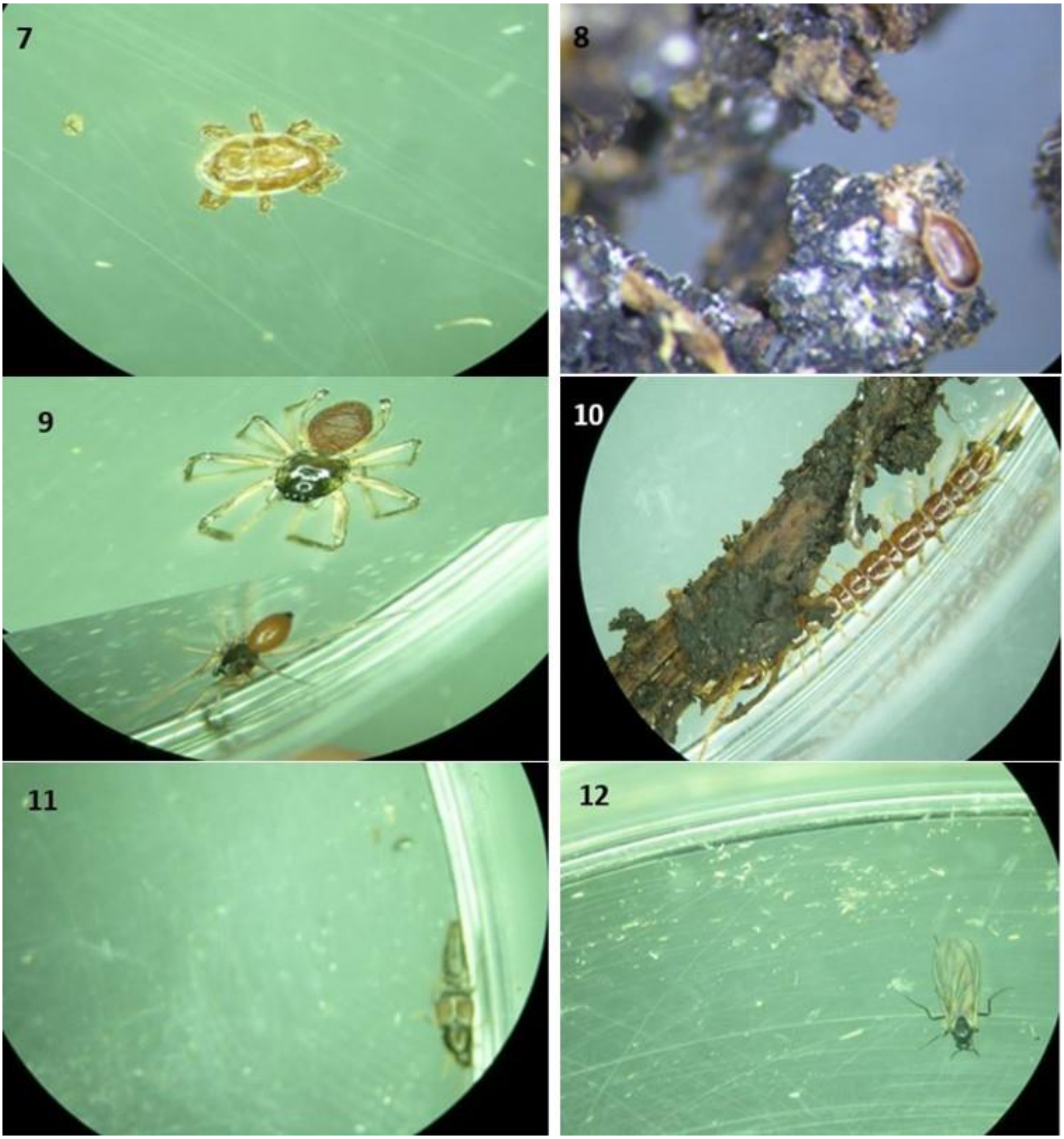

Figure 6: *Organisms in the investigated composting piles under a Stereomicroscope: 1 - Larvae of Fungus gnats: Mycetophilidae of Sciaridae, 2 - Diptera Larvae, 3 and 4 and 5 - Springtails (Collembola), 6 and 7 - Mites (Gamasina), 8 - Mite (Uropodina), 9 - Spiders (Araneae), 10 - Centripedes (Lithobiomorpha), 11 - Rove Beetles (Staphylinidae), 12 - Soldier Fly (Diptera)*

3.2.1 **Springtails** (Pictures 4 and 5 in Figure 6)

Phylum: Arthropoda; **Sub-phylum:** Hexapoda; **Class:** Entognatha; **Order:** Collembola

Springtails were found in all samples; they were extremely numerous and different colours in all samples. They are very small wingless insects and can be distinguished by their ability to jump when disturbed. They are usually less than 5 mm and the smallest do not exceed 1 mm. Surface species are often brightly coloured (blue-violet, green, grey-brown, etc.), while deep-coloured species are colourless, white (Mršić, 1977). They run in and around the particles in the compost and have a small spring-like structure under the belly that catapults them into the air when the spring catch is triggered. They chew on decomposing plants, pollen, grains, and fungi. They also eat nematodes and droppings of other arthropods and then meticulously clean themselves after feeding. They are primarily detritivores and microbivores thus help in controlling overgrowth of microbial population in compost (Mršić, 1997).

3.2.2 **Mites** (Picures 6, 7 and 8 in Figure 6)

Phylum: Arthropoda; **Sub-phylum**: Chelicerata; **Class:** Arachnida; **Sub-Clas:** Acari; **Order:** Mesostigmata; **Suborder**: Monogynaspida; **Infraordo:** Gamasina and Uropodina

Mesostigmatas were found in all compost samples and were extremely numerous. Mites were the second most common invertebrate found in all our samples of composts. They have eight leg-like jointed appendages. Some can be seen with the naked eye and others are microscopic. Some scavenge on leaves, rotten wood, and other organic debris. Some species eat fungi, yet others are predators and feed on nematodes, eggs, insect larvae and other mites and springtails. Some are both free living and parasitic. One very common compost mite is globular in appearance, red-orange in colour- Mesostigmata and have been found in all composts- A, B and C.

Adult epedaphic Gamasina are large (ca. 0.8–1.5 mm) and long-legged, well sclerotized. They hunt on the soil surface in the litter layer for other small arthropods, particularly Collembola. Gamasina do not change soil structure or plant productivity directly. However, as predators, they influence population growth of other organisms and thereby have an indirect effect on overall ecosystem performance. In the last few decades, they have gained an increasing interest in the context of bioindication, pests and pest control, decomposition, and human health. Because of their abundance, species richness and distribution as well as from their position in the web of interactions within the agroecosystem, Gamasina are good indicators of soil conditions, ecological disturbance and anthropogenic impact (Koehler, 1999).

3.2.3 **Centipedes** (Picture 10 in Figure 6)

Phylum: Arthropoda; **Sub-phylum**: Myriapoda; **Class:** Chilopoda; **Order:** Lithobiomorpha

Centipede is fast moving predator. There were a few of them found in the top few inches of the compost pile B. The species is app. 15 millimetres long and 1 millimetre wide. It is pale brown in colour with 15 pairs of legs. Centipede have formidable claws behind their head which possess poison glands that paralyze small red worms, insect larvae, newly hatched earthworms, and arthropods mainly insects and spiders. Controls insect growth in compost. Centipedes are sensitive to pollution, mainly affected by the bio-accumulation of individual pollutants, especially metals-zinc, cadmium, copper (Mršić, 1997).

3.2.4 **Spiders** (Picture 9 in Figure 6)

Phylum: Arthropoda; **Sub-phylum**: Chelicerata; **Class:** Arachnida; **Sub-Clas:** Acari; **Order:** Araneae

Spiders were present in sample B and C, but not in the sample A. Spiders are attracted by ready source of food such as invertebrates in composts. Most spiders in compost are harmless and work as scavengers (Mršić, 1997).

3.2.5 **Soldier fly** (Picture 12 in Figure 6)

Phylum: Arthropoda; **Sub-phylum**: Hexapoda; **Class:** Insecta; **Sub-Clas:** Pterygota; **Order:** Diptera

Soldier fly was present in the samples of the composting piles B and C. They are an important animal group for pedogenetic processes. This is especially true for larvae, as adult animals do not live in the soil. Larvae are saprophagous scavengers, voraciously eat the debris, while the adult fly feeds upon vegetable trash. They take part in many important biological processes in soil such as the decomposition of plant litter and nutrient cycling. Soil dwelling Diptera include groups and species that vary in size as well as in food and ecological demands (Frouz, 1999).

3.2.6 **Rove beetle** (Picture 11 in Figure 6)

Phylum: Arthropoda, **Class**: Insecta, **Order:** Coleoptera, **Suborder:** Polyphaga, **Infraorder:** Staphyliniformia, **Superfamily**: Staphylinoidea, **Family:** Staphylinidae

Rove beetle was found in the compost pile B. They concentrate in fallen decomposing fruits, the space under loose bark of fallen, decaying trees, drifted plant materials on banks of rivers and lakes, and dung, carrion, and nests of vertebrate animals. They suppress populations of pest insects and mites in numerous crops (agricultural, horticultural, and forest entomology), and of biting flies (including mosquitoes), and fleas. Staphylinidae form a substantial part of the world’s biodiversity (Howard, 1999).

3.2.7 **Larvae of fungus gnats:** Mycetophillidae (Picture 1 in Figure 4) and **Diptera Larva** (Picture 2 in Figure 6)

Larvae of Fungus Gnat was present in all samples of compost. Diptera Larva was found in the composting pile B. About 75% of all insect species go through the four stages of complete metamorphosis - egg, larva, pupa, and adult. The larva is a specialized feeding stage that looks very different from the adult. Fortunately, there are just a few basic larval types and they are relatively easy to recognize. Thicker thoracic legs, a more box-shaped head, and wider abdomen. Fungus gnat larvae resemble midge larvae but do not have fleshy legs. They live in moist, decaying organic matter, especially accumulations of fallen leaves or dead grass (Mršić, 1997).

4 CONCLUSIONS

Mesofauna and macrofauna comprise diverse organisms of various shapes and sizes right from tiny mites to large insects in the investigated composting biomass. Their role is to crush/break down organic material that than provide nutrients for the entire food web and thus restore natural balance in the compost. In the presented research, we wanted to found out mesofauna and macrofauna witch include predators, scavengers and decomposers are housed by composting hop biomass in winter.

Mesofauna and macrofauna in the composting process have a role as regulators for microbial activity and as microbial feeders. The fauna abundance in our investigated composting piles after five months of the composting process star, in January 2022, was dominated by Springtails, Mites, Spider, Centipeds, Soldier flies, Larves. The diversity of mesofauna and macrofauna in this stage was higher in the composting piles B and C compared to composting pile A. In the compost pile A, there were just springtails, mites and larvae. This could be attributed to higher temperatures (too high obviously) in the thermophilic phase.

Small particle size, through its effect on increased surface area, encourages microbial activity and increases the rate of decomposition, potentially the diversity of the mesofauna, macrofauna will increase; this was reflected in composting pile B.

However, the higher diversity in this pile can also be a consequence of added effective microorganisms at the composting pile formation.

In this stage of composting process, numerically the most abundant in all composting piles were Springtails and Mites.

A deeper understanding of diversity of fauna in compost mixtures of hop waste biomass is necessary, in order to assess the effect of composting which may further improve soil structure. We would like to gain mature compost, rich in nutrients, safe and also rich in microorganisms and in fauna. The composting hop biomass contained invertebrates in this phase majorly Arthropods witch are helpful for the formation of quality compost. There was the most diverse presence of Springtails, Mites, Spiders, Centipeds, Soldier flies, Rove Beetle, Larvae in the composting pile with added effective microorganisms EM at the start of composting. In the pile with added biochar, the diversity was the lowest among investigated piles; we found only a Springtail, Mites and Larvae. This was by our opinion the circumstance of very high temperatures, that appear in this pile during the thermophilic phase. In the pile with no additive, the diversity was almost comparable with composting pile with added EM; we found a Springtail, Mites, Soldier flies, Spiders and Larvae. We will repeat the investigation when the composts will be mature, finished.

Arthopods are an important part of the complex ecosystem that is required to decompose organic waste. It is important that the structure of this food chain keeps different populations (meso, macrofauna) under control, maintaining a healthy and balanced compost pile. We anticipate that the number and diversity of organisms will increase by the end of the composting process, which will be a matter of our further research. However, already on this stage it can be suggested that compost from hop waste biomass is a niche for diversity and the abundance of compost mesofauna and macrofauna.

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