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SAFE

Sustainable water reuse practices improving safety in agriculture, food and environment

Deliverable: D2.2

Reports on sampling protocols and evaluation of soil biological quality

Work Package: WP2

TASK2.2 Evaluation of soil biodiversity

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Brief Description	The Soil Biological Quality Index evaluates soil alterations considering the edaphic populations. The soil fauna is composed of organisms with different sensitivities to alterations of natural or anthropogenic origin and to the chemical-physical balances that characterize the soil itself. UNIBAS provided the other units with an operational plan for measuring soil biodiversity based on the identification of the soil microarthropod community. UNIBAS also provided the other units with an efficient operational plan to increase the abundance of beneficial arthropod fauna and biodiversity by incorporating flower strips with perennial plants with long flowering periods in the demonstration sites. Natural enemies and pollinators on flower strips and crops will be evaluated based on the sampling protocols implemented at UNIBAS.					
Keywords	Soil, biodiversity, microarthropods, QBS-ar, insects, floral stripes, experimental fields.					

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EXECUTIVE SUMMARY

PRIMA-SAFE focuses on smallholders' farms in Mediterranean areas affected by water scarcity, farms providing in some areas from 60 to 80% of the food and, due to the increase of the world population, under growing pressure especially in rural poor regions. PRIMA-SAFE will optimize innovative water reuse strategies, ensuring their safety both for environment and human health and more sustainable agriculture production systems, through an integrated water perspective. PRIMA-SAFE will: a) develop, validate, and optimize novel low-cost and low-energy urban decentralized wastewater treatment and reuse; b) evaluate their impact on crops performance, using modern -omics tools, and on local biodiversity; c) monitor emerging pollutants in water, soil, and produced crops and evaluate the safety of the proposed approaches; d) minimize the impacts and promote environment respectful practices, like pest management by biofertilizers; e) locally promote farmers' acceptability.

In this framework and among other activities, UNIBAS is responsible for the Deliverable D2.2, entitled "Evaluation of soil biodiversity", part of WP2 (task 2.2), which is connected with WP1 (field monitoring of selected contaminants and pathogens) and WP3 (T3.4: Solution for increasing biodiversity).

Unibas is involved in the preparation of an operational plan to measure the impact of water treatment technologies on the biodiversity of the soil microarthropod community.
Activities of the first 12 months of the project (**Evaluation of soil biodiversity**):
- Implementation of the QBS-Ar Method.
- Development of an operating plan for measuring the impact of water treatment technologies on the biodiversity of the soil microarthropod community.
- Reports on sampling protocols: explanation of the procedure and information on the necessary materials.

The Soil Biological Quality Index called QBS-Ar evaluates soil alterations, considering the edaphic populations present in it. The soil fauna is composed of organisms having different sensitivity to alterations of natural or anthropic origin and to the chemical-physical balances that characterize the soil itself: these organisms are therefore considered good indicators. Soil microarthropods are insects (Collembola, Isopoda, Protura, and Diplura), arachnids, crustacean and myriapods. Relative low values of the QBS-Ar index will be indicative of degraded environmental conditions, while higher values will be indicative of good environmental conditions.
UNIBAS provided the other units the procedure and the information on the necessary materials. Samples from Greece, Morocco, Tunisia and Algeria will be sent to UNIBAS for the determination of microarthropod biological forms, assignment of the Ecological-Morphological Index (EMI) and QBS-ar (Soil Biological Quality-arthropod) index computation.

Unibas is also involved in the preparation of an operational plan to measure the increase in the abundance of the useful arthropod fauna and biodiversity by the inclusion of floral stripes with perennial plants having a long flowering period (such as *Calendula officinalis*, *Echinacea purpurea*, Tagetes sp.) in the demonstration demo sites. Natural enemies (parasitoids, mirid bugs, ladybugs) and pollinators (honey bees and wild pollinators) on different flower strips and crop will be evaluated based on sampling protocols implemented at UNIBAS.
Activities of the first 12 months of the project (**Solutions for increasing Biodiversity**):

- Implementation of the methods for measuring biodiversity.
- Development of an operating plan for measuring the impact of water treatment technologies on the arthropods biodiversity and insect pests.
- Reports on sampling protocols: explanation of the procedure and information on the necessary materials.

The inclusion of floral strips with perennial plants having a long flowering period (such as Calendula officinalis) within fields would increase abundance of the useful arthropod fauna and biodiversity (T3.4: Solution for increasing biodiversity).

UNIBAS provided the other units the procedure and the information on the necessary materials. When necessary, samples from Greece, Morocco, Tunisia and Algeria will be sent to UNIBAS for the determination of the insects.

The impact of water treatment technologies on the growth of the different floral stripes will be also evaluated based on agronomic measures (biomass, number of stems and flower heads per plant).

SAMPLING PROTOCOLS FOR EVALUATION OF SOIL BIOLOGICAL QUALITY

Soil samples will be extracted from field studies (Greece, Morocco, Tunisia and Algeria) during two sampling periods: at the beginning and end of cultivation. The following material is required for the extraction of microarthropods (**figure 1**):

- A shovel (or, preferably, a hand auger).
- Mesh-covered funnels (or a sieve inside a funnel).
- Plastic jars.
- Incandescent lamps (or a light and heat source close to each other).
- Ethyl alcohol (preferably colourless).

Figure 1. Material required for the extraction of microarthropods.

Therefore, Berlese-Tullgren funnels (**figure 2**) should be built (one for each sample).

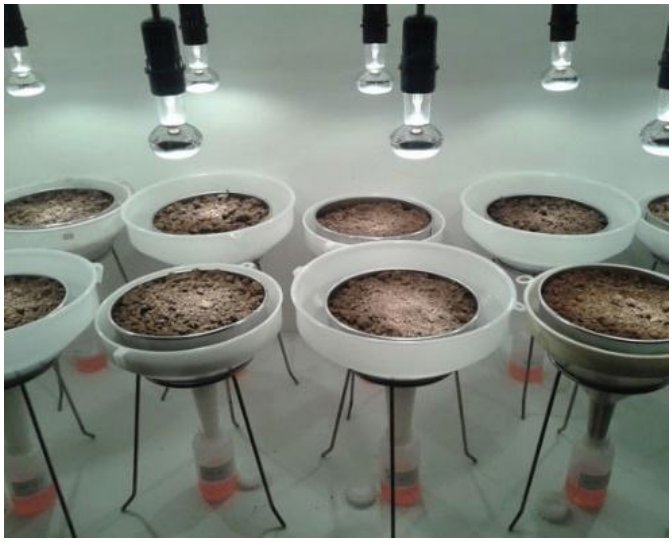

Figure 2. Examples of Berlese-Tullgren funnels.

A soil sample should be composed of three soil clods (10 x 10 x 15 cm depth), which will be taken at 3 points in each experimental plot using a hand auger. Each sample is placed in a plastic bag, kept in darkness at 5°C and transported to the laboratory for arthropod extraction, taking care to leave an air reserve that allows the (live) organisms to breathe (**Figure 3**). Three replicates per treatment.

Figure 3. Soil samples.

Microarthropod extraction is carried out by gently placing soil clods on mesh-covered funnels (mesh 2mm, 20 cm in diameter). A plastic jar containing 50 ml of hydroalcoholic solution (70%) is placed at the bottom of the funnel to store the extracted arthropods. Incandescent lamps (40 watts) are placed 20 cm above the soil clods **(figure 4**). The extraction time is 14 days.

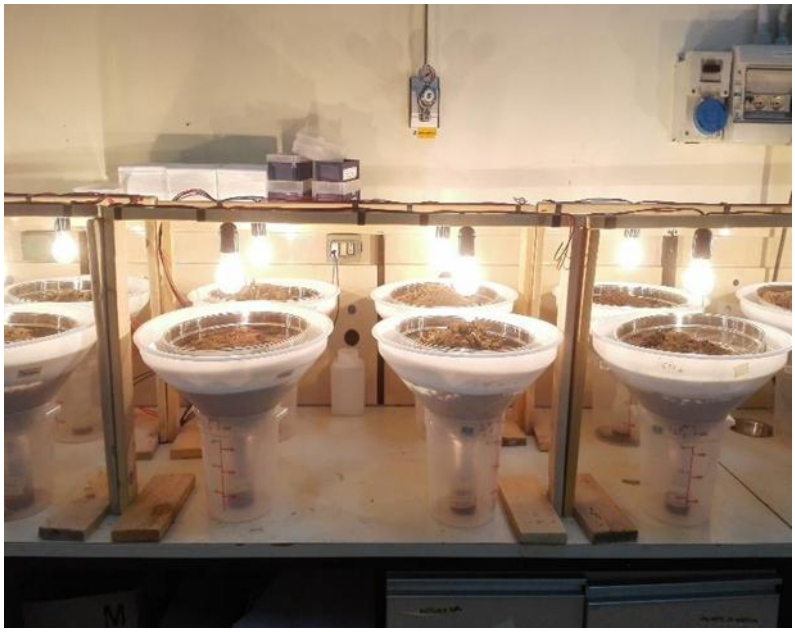

Figure 4. Microarthropod extraction.

Lamp, which produces heat, gradually desiccates the soil (**figure 5**). The insects, escaping from light and desiccation, lurk deeper and deeper into the clod until they fall into the funnel.

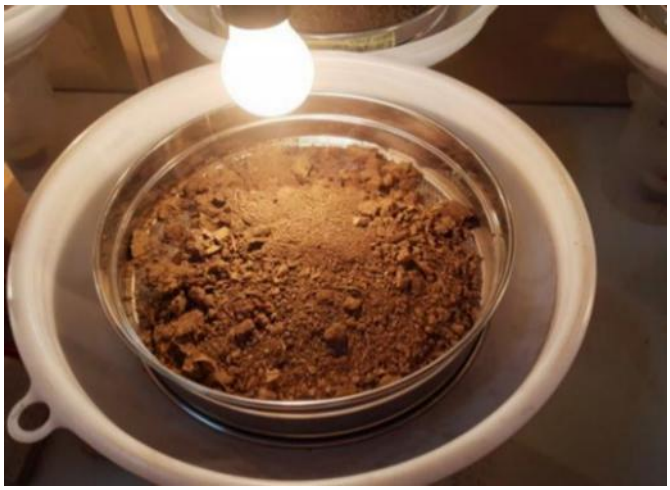

Figure 5. Incandescent lamp.

The arthropods in the plastic jar will be gently separated from the fallen soil and transferred to new containers filled with clean alcohol.

The extracted specimens will be observed under a stereomicroscope (**figure 6**), the biological morphs will be determined, and the Ecological-Morphological Index (EMI) assigned based on a reference table that considers the degree of adaptation to the edaphic environment. If necessary, the extracted specimens will be sent to UNIBAS for identification.

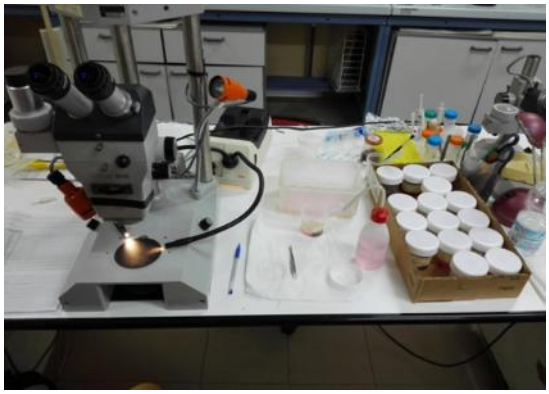

Figure 6. Arthropods identification

Finally, the QBSar index will be computed as the sum of the EMI values and any differences between treatments will be analyzed.

SAMPLING PROTOCOLS FOR INCREASING BIODIVERSITY: FLOWER STRIPS

An increase in the abundance of the useful arthropod fauna and biodiversity could be obtained by Inclusion in the experimental fields of floral stripes with perennial plants having a long flowering and vegetative period (**figure 7**). The choice of the plants is linked to the local conditions of the demonstration demo sites.

Figure 7. Examples of floral strips placed in an experimental field.

Arthropods will be sampled using pan trap sets consisting of one blue, one yellow, and one withe bowls (**figure 8**). Pan traps provide an ample return of data for relatively short periods of time and are particularly appropriate for faunal surveys. They also provide a good estimation of species richness and of the attractiveness of plants.

Figure 8. Examples of pan trap sets.

The traps will be made by painting plastic bowls (17 cm in diameter, 4.5 cm deep), with blue (RAL standard color codes: 5015) or yellow (RAL standard color codes:1023) acrylic paint sprays, or left white.

A pan traps set will be placed on the ground, as close as possible to the flower plants of each experimental plot / treatment.

Each trap will be filled with 400 mL of water and 4 mL of dishwashing detergent to break surface tension.

Traps will be set out early in the morning and collected three days later, at the same time. In case of rain or accidental spillage, pan traps will be removed and the sampled specimens will not be considered; the traps will be replaced 24 hours after rain stopped.

The arthropods will be removed from the soap-water solution using a fine mesh colander and gently transferred with a soft paintbrush in 50 ml Falcon tubes, filled with 70% ethanol (**figure 9**). Falcon tubes will be stocked at 4°C until the identification of the arthropods.

Figure 9. Pan traps sample collections.

If necessary, the extracted arthropods will be sent to UNIBAS for identification at order, family, and, when possible, at the species level.

CONCLUSIONS

This deliverable dealt with the generation of sampling protocols for the evaluation of arthropods biodiversity for the partners involved in the SAFE project. This deliverable also dealt with the generation of sampling protocols for the increase abundance of the useful arthropod fauna and biodiversity by the inclusion of floral stripes with perennial plants having a long flowering period.
The development of an operating plan for the measure of the impact of water treatment technologies on the biodiversity of the soil microarthropods community is given in this report. Samples of soil from Greece, Morocco, Tunisia, and Algeria will be sent to UNIBAS for the determination of microarthropod biological forms, assignment of the Ecological-Morphological Index (EMI) and QBS-ar (Soil Biological Quality-arthropod) index computation.
This report also provided an efficient operating plan for the increase abundance of the useful arthropod fauna and biodiversity by the inclusion of floral stripes with perennial plants in the experimental field. For the floral strips, necessary optimizations will be carried out for the choice of the plants since it will be linked to the local conditions of the demonstration demo sites.