POREM – Project LIFE17 ENV/IT/000333

“Poultry manure based bioactivator for better soil management through bioremediation"

HANDBOOK

New strategies for better soil management via remediation with poultry manure based bioactivator

This handbook explains (through text, boxes, photos, pictures, graphs and charts) the technical and operational characteristics, protocols and procedures for POREM bioactivator production from poultry manure/litter, as well as technical and environmental advantages. It also contains instruction for its use, issues related to its application procedure for crop production and land restoration of European degraded soils, such as in Italy, Spain and Czech Republic.
This handbook is the results of three years of experience matured by the 6 beneficiaries, public and private, in 3 European countries, expressing different and integrated climatic and production needs. Applications were in Italy, both the Po Valley and Mediterranean areas, semi-arid areas in Spain and continental areas in Czech Republic during POREM LIFE project.

This handbook is addressed to Public institutions involved in restoring areas characterized by degraded and organic matter-depleted soils; this guideline has the ambition to provide an instrument for national and European policy makers as well as to the general public, because the demonstration of the POREM system will contribute to create and spread new perspectives and possibilities in the field of land use and management.
As well as, the handbook explains a further application for poultry by-products, in a circular economy vision.
Specific guidelines for POREM bioactivator production and use were compiled; they are addressed to farmers, poultry farmers, organic fertilizer manufacturers, gardeners and companies that care for parks and gardens (see reference).

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List of key-words and abbreviations

ABP: animal by-product
MAP: magnesium ammonium phosphate
MDS: Manure Drying System
PM: poultry manure (often used as general category to include poultry litter from chicken and broilers, poultry dejections from laying hens, poultry litter from turkeys, …)
VAP: Vegetable Active Principles

1. ABSTRACT

POREM BIOACTIVATOR (referred hereunder also as POREM) is a sustainable fertilizer for degraded land, based on natural resources: it is a product obtained from poultry manure amended with a natural enzyme preparation derived from plants (**VAP**[1]) and represents a continuous source of organic matter and nutrients for soil restoration, taking advantage of the main poultry productions waste. The process of POREM production is easy: it takes 90 days, during which the animal waste, stowed in heaps, is inoculated with the VAP enzymes triggering the stabilization of the material; bio-activator stability is correlated with maturation time. The animal waste piles must be protected from atmospheric events, i.e. set in an agricultural barn or shed, neither moved nor mixed during the whole period. During the maturation process, in the waste matrix, thanks to the effect of plant enzymes, a microbial flora is selected that is able to stabilize the different nutritional elements present. In this process, the poultry manure maturation leads to the release of Magnesium (Mg) and Phosphorus (P), which react with the Ammonium (NH_4^+) present in this material, mainly forming struvite (hydrated Ammonium and Magnesium phosphate $(NH_4)\ MgPO_4\ 6(H_2O)$), having low water solubility and considered a slow-release fertilizer. The first effect of this reaction is the fixation of Ammonium, which leads to a reduction in the odor emissions of the final product. This means that the POREM BIOACTIVATOR has little *Nitrogen* (N) losses when it is incorporated into the soil as fertilizer, affecting positively soil organic matter levels. **All these mentioned factors (*Nitrogen* availability, sustained supply of stabilized organic substances, and low odor impact) place POREM on the leading edge of the fertilization industry,** providing solutions for the amendment and regeneration of soils, both agricultural and natural, both of private and public interest. The final product is a semi-solid material (shovelable) ready to be used as is, or pelletized to facilitate packaging, storage and transport. Once the POREM bioactivator has reached the maturity it maintains its stable characteristics over time.

[1] Vegetable Active Principles or VAP, natural enzyme preparation from plants belonging to the Cucurbitaceae, Graminaceae, Labiatae, Apiaceae and Rutaceae families or parts thereof, according to EU patent 1314710 (**A process of maturing and stabilizing biomasses under reduction of smelling emissions**).

2. INTRODUCTION

IMPORTANCE OF SOIL FOR AGRICULTURE

Soil is a natural resource, essential for life on our planet; we have an obligation to protect and preserve it for present and future generations. The soil is a complex matrix, made up of mineral matter, organic matter, air, water and living organisms (worms, algae, bacteria and fungi), combining to carry out physical, chemical and biological processes. The soil is not a static but a dynamic entity; it is a living entity, with continuous synthesis and degradation processes derived mainly from the activity of its microbial populations. There is therefore a vitalist conception of soil, but neither scientists nor administrations have been able to transfer to society the importance of soil as a natural resource, necessary for life. Its degradation is a serious threat to the future of humanity and of all living beings in general. Today we know that the soil is not only the basis for agriculture, but also that life depends on it.

The importance of soil in agriculture is indisputable; contributes to different ecosystem services such as water flows and the increase in stable C in that soil, thus mitigating climate change. But due to various anthropic actions, effects derived from climate change such as major droughts or excess greenhouse gases, will undoubtedly influence the soil and the agriculture that develops on it. Hence the interest in adapting Mediterranean agriculture to the aforementioned climate change, avoiding negatively affecting the soil and its degradation through appropriate management and strategies.

The soil and its processes condition agriculture, affecting its productivity, functionality, and therefore, its fertility. Agricultural land must be considered as the core of future sustainability, hence the interest in maintaining good health and quality. A paradigm shift is proposed: not only fertilizing the plant to obtain immediate agricultural yields, but also focusing our interest on the soil and its edaphic fertility, capable of producing positive effects for the plants and the associated environment. Europe is committed to this line, through programs such as “EJP SOIL” that have been launched, where the soil takes center stage for the agriculture of the future; or the new European pillars for "EUROPE HORIZON 2021-2027", where one of them is "Soil Health and Agriculture", its motto being: "caring for the soil is caring for life".

AGRICULTURAL SOIL: SUSTAINABILITY, HEALTH AND CLIMATE CHANGE

Soil, including that used for agriculture, constitutes the largest terrestrial organic carbon reserve, more than double the amount stored in vegetation. We know the importance of capturing CO_2 from vegetation, but even more important would be to increase the capacity as a sink for C of our soils, whether agricultural or not. In addition, these soils help provide benefits to the ecosystem, such as providing drinking water, avoiding desertification, or providing resilience against floods and drought; Soil mitigates climate change by sequestering carbon and reducing greenhouse gas emissions.

Soil is the basis for agriculture and the substrate for natural ecosystems across the planet. Conventional management practices such as plowing, cultivation patterns, as well as the use of pesticides and fertilizers, have had an influence on the quality of water and the atmosphere, since they have generated changes in the capacity of the soil to produce and consume. gases such as CO_2, nitrous oxide, and methane. With regard to CO_2 as a typical greenhouse gas, agricultural vegetation plays an important role in capturing CO_2 from the atmosphere within its photosynthesis process, including it in its system and generating biomass; Part of said biomass can be incorporated into the soil, originating, on the one hand, the microbial activity that degrades said plant residues and emanates CO2 into the atmosphere, but part of said carbon from the plant remains will be incorporated into the soil in a fairly stable way (carbon sink),

One aspect to highlight is that, in agriculture, defective and sometimes irrational anthropic actions have promoted the loss of organic matter, reducing fertility, negatively altering different properties of the soil, also increasing erosion and atmospheric CO_2; consequently, inappropriate agricultural practices contribute to global warming of the planet. The high levels of gases associated with the greenhouse effect and the alterations in the hydrological cycles have produced changes in the global climate, as well as the reduction of ozone. To face this threat, it is required, on the one hand, to understand what is happening with a natural resource such as the soil, and how the agricultural management of said soil is affecting its processes; on the other, to propose alternatives for use that maintain both environmental quality and efficiency, that is, sustainable alternatives.

It should be noted that, in Mediterranean agriculture, the use of organic amendments or organic fertilizers is increasingly necessary to improve the quality and health of the soil, in addition to increasing its organic matter and stable C; These organic amendments will increase the water retention capacity of these soils, which is an added value in the fight against climate change.

FERTILITY AND MICROBIAL BIODIVERSITY OF AGRICULTURAL SOILS WITH ORGANIC AMENDMENTS INCORPORATION

In theory, the addition of organic matter is carried out by the constant recycling of it, in the form of residues of living or dead plants and animals. However, in large part the soils of vulnerable zones, the C inputs are insufficient since the balance between the C inputs from the vegetation and the mineralization rate produced by the microbial activity of the soil is negative. In these areas, mineralization processes predominate over humification or C fixation, a common characteristic in these soils being the low content of organic matter and its exposure to degradative processes.

Degraded soils need strategies to reduce erosion and remedy the effects of degradation. Soils in semi-arid areas have very low microbial activity, low levels of microbial biomass and low content of organic matter (0.5-2%). If the characteristics of degraded soils in semi-arid areas are recovered and improved with the application of organic amendments, the cycle of nutrients (Clark et al., 2007; Hernandez et al., 2015) and their quality in general will be improved. As previously indicated, the degradative processes in soils affect natural soils, and in particular, agricultural ones; soils lose organic matter, and this affects their fertility and functionality, as well as the biodiversity of said soil itself (remember that organic matter acts by providing substrates to microbial populations, and is an important part for the functioning of microbial populations that are found in soils). To recover organic matter, one way that should be considered is to propose exogenous contributions that are capable of building new organic matter in the soils. However, this is not easy because the behavior of exogenous organic matter and its ability to influence the generation of new stable organic matter in soils will depend fundamentally on the quality of said organic matter, and on the receiving soil, cultivation management and climate.

When it comes to adding "organics" to the soil, we must bear in mind if this is done in order to increase the nutritional contents of the soil fundamentally (organic fertilisers, potential substitutes for mineral fertilisation, in the knowledge that the nutrients are provided gradually, depending on the possible mineralisation of the organic matter), or are intended to improve soil health in general, increasing carbon and organic matter content, and achieving slow but consolidated improvements, with soil properties as varied as the physical, chemical, biological, biochemical and microbiological properties.

As exogenous organic materials capable of acting as good organic amendments for soils, organic waste such as manure, various agricultural waste, urban organic waste such as domestic waste, biosolids (although these have many problems of incorporation of pollutants, and have various limitations), organic byproducts of different agroindustrial types, organic waste conveniently treated by composting, for example (compost), and more recently, waste such as biochar (derived from pyrolysis processes of different organic waste), can be used as amendments to increase soil fertility, since while they provide nutrients to the crop, they improve the quality of the soil. These organic materials can partially or totally replace, depending on the residue and the requirements of the crop, the synthetic fertilizers used in the crop, while increasing the total organic matter in the soil (Hernandez et al., 2014, 2016, 2018).

However, here too, it must be based on a clear premise: the exogenous organic matter that is contributed to the soils must be of "QUALITY"; This means that the sources of exogenous organic material that reach the soil must be hygienically healthy (without pathogenic microorganisms), without problematic content in heavy metals, salinity, or other emerging pollutants of anthropic origin, including microplastics), and with an acceptable stability of its organic matter, so that it can have a favorable effect on the physical, chemical and biological soil properties. The soil is a natural resource that must be conserved and protected, in particular its biodiversity. It cannot be a place where any organic matter is added due to the fact that it has carbon and nutrients; It must not contain toxic elements that could put the health and biodiversity of the soil, or any of the ecosystems that could be affected at risk. Sustainability criteria must be maintained, and for this, the quality of organic materials used as fertilizers or amendments is essential.

3. INSTRUCTION FOR POREM BIOACTIVATOR PREPARATION.

3.1 Raw materials

Raw materials for POREM bioactivator production are poultry manure, poultry litter and dejections (PM in the following), always used in agriculture but with a few drawbacks.
Raw materials are the by-products of poultry farming, intensive or not, part of the production chain for eggs or for broilers, as well as for turkeys (for meat), ducks (for meat, foie gras or eggs). The value of the eggs and poultry products in the EU-27 in 2012 was 30,748 million € (BREF, 2017).

Two pillar features of raw materials for POREM production are:

- ❖ The manure has to be in **solid phase**, avoiding leachate formation and allowing the heap/pile management in barn; therefore, laying hens dejections have to be pre-dried. The dejections can be subjected to different drying systems (MDS, drying belts, …);
- ❖ The raw materials have to be **antibiotic free**, in order to prevent their diffusion to the environment. Therefore, the manures can be taken from antibiotic free poultry chain, from organic poultry farming, or raw materials can to be checked to be pests free (in Italy negative to Inhibitors search[2]).

PM characteristics depend on animal, and also on nutrition and rearing conditions; also manure management system impacts on raw material features. Due to the diversity of the starting materials, the properties of the POREM final products will fall within a range for different parameters.
Common characteristics on wet basis (when all of them dried, with Dry Matter > 50%) are:
an organic substrate, with organic carbon content 25% to 30%, rich in macronutrients (N, P, K) containing all micronutrients, rich in microorganisms useful for soils. If fresh, a rapid mineralizing N (ureic form) is present, making it a prompt fertilizer.
Various kinds of PM were tested to produce POREM bioactivator and subsequent soil application during the project (Figures 1 ÷ 4):

- Laying hen dejections after drying with manure belts (Calabria, It),
- Poultry litter from organic farming (Apulia, It),
- Laying hen dejections in Spain,
- Laying hen litter from organic farm in Czech Republic.

[2] The method allows to detect the presence of antibacterial substances in feeds, meats, eggs and any other matrices (Decreto Ministeriale 10.03.97 All IX).

Fig. 1 Raw material (conventional) in Czech Rep.: laying hens litter (source: 2020 Mistřice - EPS)

Fig. 2 Raw material (organic) in Italy: broiler litter from organic rearing (source: 2020 Foggia - ASTRA)

Fig. 3 Raw material (conventional) in Italy: laying hens litter (source: 2020 Cesena - ASTRA)

Fig. 4 Raw material (conventional) in Spain: broiler litter (source: 2020 Murcia - MICRONADIR)

3.2 Equipment

The required equipment is connected to:

- The shed/barn where to collocate the POREM preparation, with a roof cover to prevent wetting the poultry manure/litter, better if paved (concrete, ….) for machinery operation, and well ventilated; the roof can be fixed or removable;
- The equipment necessary for handling solid raw manure and mature POREM (loader / tractor with shovel, or bucket);
- In some cases, for example for commercial production, temperature monitoring devices are required.

3.3 Operational procedure/ treatment procedure

Preparation of heaps for the POREM bioactivator production, under a roof, fixed or removable. The procedure was developed for pilot- and small-scale production (EP 1314710).
The heaps have to be prepared in a well ventilated, roof-covered space. Plastic sheet pile-covering with limited ventilation (laid directly on the manure pile surface) is to be avoided because of water condensation on POREM. The objective is to maintain the internal heap humidity in a range optimal for microorganism activities, but lower than 50% – the threshold for ammonia emissions from poultry dejections The required material proportions for the POREM production are listed.

- **Unit dosing**: 1000 kg of poultry manure + 4 kg VAP (a precautionary value for 60-90 days' maturation)

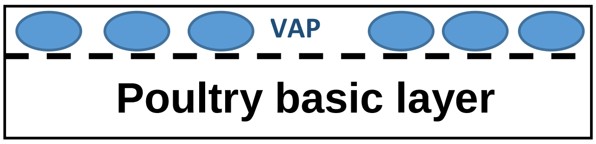
<u>Preparation</u>:

a. Select an area suitable for the heap set up: consider accessibility for processing machinery and the 90-day no heap-disturbance requirement.
b. In case of multi-heap production, make one heap at a time.
c. The method of preparation is described in Figures 5 (A, B) and 6. The treatment is performed during heap formation in three steps:[3]
    1. **Basic layer formation** Prepare the selected area. Prepare the base **poultry manure** layer from well mechanically homogenized material, under a roof, about 0.8-1 m thick.
    2. **VAP addition** (Figure 1.A) Take the VAP (natural enzyme) preparation with a doughy consistency and toss them as they are or roughly fragmented onto the heap to be treated, on the prepared layer. Add all VAP dose, inside the layer.
    Immediately cover the tossed VAP with manure (the enzymatic product is sensitive to light and air).
    3. **Heap completion** (figure 1.B) Proceed adding further poultry manure until the completion of the heap.
    (In the case of prolonged heap filling, where not all the poultry manure is available at one time to set up the heap, it is possible to add the VAP doses gradually, along the pile set up, using the same precautions; provide that, upon completion of the pile, there is the quantity of enzymatic preparation needed for the treatment of the whole pile).
d. The length of the enzyme process has the optimum of 90 days. During this period the pile must not be moved or remixed (no disturbance rule).

POREM bio-activator is stabilized after 90 days from treatment. The desirable properties last for at least 180 days.

[3] Each heap base takes about 6-8 m^2 for an amount of 3000 kg (1m high, min-max 3000 kg – 4000 kg of poultry manure for each pile). Maybe would be useful for the practical purpose pilled up one long heap about 1 m height – another way of preparation to single heaps.

A)



B)

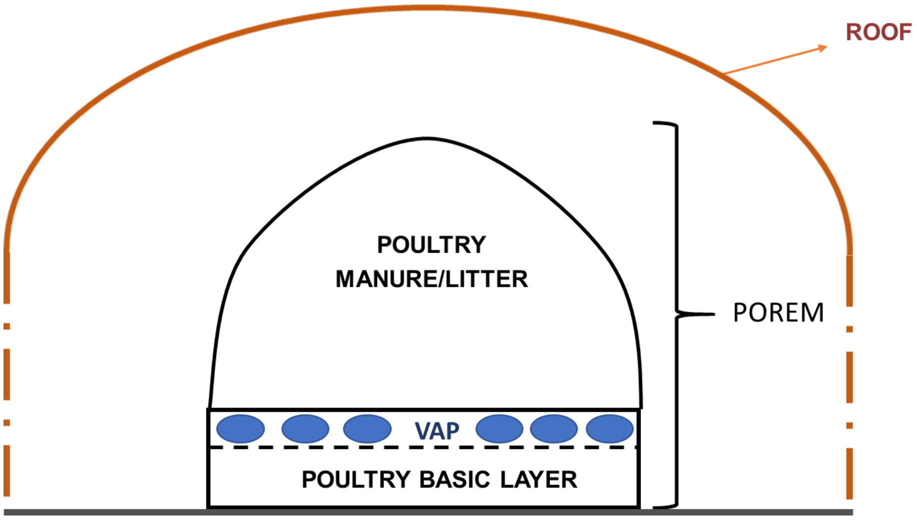


Figure 5 Preparation of POREM bioactivator: A) Poultry manure basic layer B) POREM Heap

3.4 Pelletizing and conservation

Also pelletizing strategy has been realized.

A preliminary drying phase is necessary to have a humidity < 30%.

- POREM can be placed in stacks in the storage area, in the air and in the sun, for a better drying of the material;
- after the positioning of POREM in the ventilated shed, a further phase of drying follows in order to have a humidity < 30%

Bulk dried POREM bioactivator was transformed into pellets by an extrusion process carried out by an element rotating (pressure roller), contained within a cylindrical element (compression die), which compresses the introduced material and determines its escape through special perforated matrices obtained along the walls of the die.
During this operation, a progressive densification of the material that comes out is determined in the form of small cylindrical elements. Knives, placed on the outside of the die, cut the pellets in length desired. During the extrusion phase, temperatures above 90°C can be reached.
A final stage of cooling, during which further drying of the pellets and the separation of the fine parts takes place, is indispensable for stabilizing and forming the small cylinders.
Subsequently, POREM bioactivator can be packed, both in big bag and in smaller packaging bags. Samples are shown in Figure 7.

	
Pre-piling poultry manure homogenization	Make sure the accessibility of the area by vehicles
	
Fast non-contact VAP application with immediate coverage of the second part of poultry manure	
	
VAP packed per 1 kg	Heaps 3000 kg of poultry manure each

Figure 6. Formation of a 3 tons pile for POREM bioactivator production

Figure 7. Sample of pelletized POREM bioactivator (source: pilot test 2020 – SOLDANO)

4. POREM BIOACTIVATOR CHARACTERISTICS

The POREM bioactivator has been obtained from poultry manure with the addition of VAP[4], subjected to a "no-disturbance composting" process, that is a process of substrate maturation, subject to steep oxygen gradient from surface crust (aerobic) into the interior (micro-aerophilic, up to local temporary anaerobic/anoxic). It takes about 90-day without movement, carried out according to established protocol).
The bioactivator should have the following qualities (range for some parameters):

- Its content in organic matter (in organic carbon) is high (around 30%), which gives it a clear character of "soil bioactivator useful to increase soil organic matter", with the capacity to recover soils subject to erosive processes.
  A remarkable aspect of the POREM bioactivator is that its organic matter has a very active part, formed by a quantity of carbohydrates greater than 1% w/w. That indicates that it will perform high microbial activity when it has the right conditions (aerobic conditions, humidity, etc.). In addition to a part of very active and labile organic matter, POREM bioactivator also has a high, organic, water-soluble carbon (around 10%). This is indicative that this POREM product contains a good part of its organic matter (about 1/3 of its organic carbon) which is “active”); about 2/3 of its organic carbon is more recalcitrant, and can be sequestered in the soil where it is added (ecosystem service for soil sustainability).

- The nitrogen content is high (between 2.5÷4%), in greater quantity than in composts; up to 50% of this N is soluble in water, formed by an inorganic part (nitrates and ammonium), and an organic part that could be mineralized and constitute nitrogen for plants and microorganisms.
- P and K maintain high values in POREM (around 1.7% for P, and 2.1% for K); This is indicative that this product not only has the conditions to be a good organic soil amendment, with a part of its carbon stable and another active, but that it maintains appreciable values of macronutrients such as N, P and, above all, K. POREM bioactivator displays some behavior as bio-fertilizer.

- POREM bioactivator achieves very good biochemical activity with regard to β-glucosidase activity. This implies that the most labile part of the organic matter that contains the bioactivator will be decomposed, and both microorganisms and plants will be able to use energy derived from these carbon substrates.
- The urease activity is limited or absent, which indicates that there will reduce or prevent release of ammonium into the atmosphere; possibly ammonia is fixed in the organic matrix, and there is no need to synthetize this enzymatic activity.

Finally, common to many organic fertilizers/amendments, micronutrients (B, Cu, Fe, Al, Mn, Mo, Zn, Ca, S ….) are retained by the organic matrix and their solubility and bio-availability is mediated by the organic substance.

[4] Vegetable Active Principles or VAP, natural enzyme preparation from plants belonging to the Cucurbitaceae, Graminaceae, Labiatae, Apiaceae and Rutaceae families or parts thereof, according to EU patent 1314710 (**A process of maturing and stabilizing biomasses under reduction of smelling emissions**).

The main characteristics are listed in Table 1.

Table 1. Characteristics of POREM bioactivator obtained in pilot production in Spain, Czech Republic, Italy and range obtained. All percentage are w/w; they are expressed on dry mass.

PARAMETER (unit)	SPAIN	CZECH REPUBLIC	ITALY	POREM BIO-ACTIVATOR (*range*)
pH	7	7.8	6.9	**7 ÷ 8**
EC (mS/cm)	3.85	5.2	3.1	**3 ÷ 5**
VOM (% on wet basis)	72	56	78	**60 ÷ 80**
TOC (%)	30	28	34.3	**28 ÷ 35**
C water soluble (%)	10.2	9	8.1	**8 ÷ 10**
Carbohydrates (%)	1.11	1.85	1.8	**1.1 ÷ 1.8**
Total N (%)	4.35	2.63	3	**2.6 ÷ 4.4**
N water soluble (%)	1.9	1.4	1.5	**1.4 ÷ 1.9**
Total P (%)	1.9	1.44	1.79	**1.5 ÷ 1.9**
Total K (%)	2.55	2.35	1.57	**1.6 ÷ 2.5**
Total Mg (%)	0.52	0.63	0.4	**0.4 ÷ 0.6**
Total Na (%)	0.12	0.4	0.27	**0.1 ÷ 0.4**
Phosphate (mg/l)	400	330	480	**300 ÷ 500**
β-Glucosidase (μmol PNF/g*h)	4.9	0.92	3	**3 ÷ 5**
Fosfatase (μmol PNF/g*h)	43	26	10	**10 ÷ 45**
Urease μmol (N-NH4/g*h)	ND	ND	17	**ND**

EC: electrical conductivity; VOM:Volatile Organic Matter; TOC: Total Organic Carbon; WSC: Water Soluble Carbon; WSN: Water Soluble Nitrogen; PNF: ^ p-nitrophenil phosphate.

The physico-chemical characterisation (i.e. Thermogravimetric analysis - TGA, Scanning Electron Microscopy - SEM, X-ray diffraction analysis - XRD) has shown the time evolution of POREM bio-activator properties that tend to stabilise after 60 days. The mineralization also is time dependent: it grows over that time interval and the mineral compounds that are formed, related to nutrients, could become a basin of N or P for soils. The formation of struvite (magnesium ammonium phosphate, $NH_4MgPO_4 \cdot 6H_2O$ - MAP) was detected by x-ray diffractometer analysis for all test conditions: a crystalline phase constituted by Mg^{2+}, NH^{4+} e $PO_4{}^{3-}$ with ratio 1:1:1. Moreover, the observed bio-activator stability, growing with the maturation time, is a first demonstration that the simplified and static process of POREM production can be consider effective. The replicability of POREM properties, at pile and sample level, is connected to POREM process replicability and can be considered as a further demonstration of POREM process effectiveness. The detected intrinsic properties of POREM bio-activator, that is a natural product rich in C and micronutrients, make it useful for both soil and plants.

The product is obtained anyway; its quality is subordinated to a correct treatment procedure. Regarding the POREM effect on soil: tested abandoned agricultural soils with no vegetation cover (and thus subject to erosion processes) exhibit increased spontaneous vegetation, as well as soil organic carbon content. This could be partly explained by the addition of carbon from vegetable residues present in the manure. Also, some macronutrients of interest such as N and P increase their values in rehabilitated soil after POREM incorporation. A higher potential fertility due to the increase of some enzymatic activities of the C, N and P cycle, can be also observed on rehabilitated soil.

The introduction of the POREM bioactivator into the soil showed a **good water retention when mixed with soil** (water holding capacity), thus allowing reduction in water irrigation in agricultural activities and some type of soils.

In case of litter from certified organic poultry farms, POREM bioactivator can be used in crops addressed to EU organic production (Reg. 834/EC/2007; new reg. from 2021).

5. INSTRUCTION FOR POREM BIOACTIVATOR DISTRIBUTION

5.1 POREM amount as fertilizer (N-P-K)

Calculation of nitrogen (N) units is crucial, considering factors as macronutrients content in POREM bioactivator, land as well as crop requirements. On the other hand, the amount of POREM to be added as fertilizer to the agricultural land must be measured in function of N availability in the soil, the needs of the crop according to any regulations to which it is subject (integrated or organic, for example) and the legislation that regulates quantities of N that can be used in the various geographical areas (directive 91/676/EEC).

POREM bioactivator as it is (before palletization, Fig 8), with a moisture content between 40% and 25% has a nitrogen content of 2.5% and a percentage of mineralization that varies, depending on the crop cycle duration, from 40% (cultivation of short duration such as tomato, cabbage and vegetables, in general) to 80% (cultivation of annual crop, such as tree crops, cereals and arable crops, in general). The other macroelements taken into account as fertilizer are potassium (K) and rather phosphorus (P). The latter, being mainly in the form of struvite, is stable in the soil and used by plants over long periods (slow-relase). Both of these elements, in the quantities present in POREM bioactivator, generally do not cause problems of crop phytotoxicity nor pollution of soils or surface waters. The presence of struvite is an important factor, since it is considered as a nitrogen compound able to reduce environmental impact and enhance N retention.

Fig. 8 POREM bioactivator non pelletized (source: pilot test 2020 – EPS)

5.1 Soil application of the product

POREM bioactivator (no pelletized) is distributed to the soil before sowing or transplanting, using the means provided by the farm, such as a common fertilizer spreader (conventional farmer machinery), then applied through mechanical incorporation. The distribution task is even easier with the pelletized product.

A burial in the surface layer, such as a passage with a surface harrow, is recommended after the POREM distribution; according to the incorporation practice common to all products of organic origin.

In the case of several crop cycles, the administration of POREM bioactivator can be repeated in a reduced quantity, calculated following the instructions given in paragraph 5.1.

The fertilizer distribution would be desirable for at least two consecutive years, with the aim of favouring the regenerative capacity of the soil of the POREM bioactivator. In addition, its intrinsic and slow-release characteristics ensure the availability of nutritional elements for relatively prolonged periods, without excess of nutrients and limiting losses of various types.

Fig. 9 POREM loading and application in Italy (source: 2019 pilot test in Apulia region - ASTRA)

Fig. 10 POREM loading and application in Czech Republic (source: 2020 pilot test in Mistřice – EPS)

6. EXPECTED RESULTS ON CROP PRODUCTION

Using POREM bioactivator it is possible to obtain a quantitative and qualitative improvement of yields obtained from crops grown on treated soils: the field tests proved a significant sugar content (°Brix) and coloring of the berries of industrial tomato, an increase in the quantitative yield in cabbage, a “starter effect” in terms of superior vigor up to harvest on both cereals and vegetables, compared to the untreated plants.

Through the use of POREM bioactivator it is possible to drastically reduce the Nitrogen (N) intake (up to about -69% of N, as resulted in the pilot field tests), obtaining the same results on both quantitative and qualitative terms. This prerogative makes this product particularly suitable in vulnerable areas due to the EU Nitrates Directive (91/676/EEC).

POREM bioactivator brings quality organic matter to the land, that helps to improve the soil from the point of view of structure and permeability and, above all, of water capacity, characteristics that are often limiting in degraded soils. The organic matter is naturally also a source of Carbon (C), necessary for soil microorganisms.

This leads POREM bioactivator to be particularly suitable for the regeneration of degraded soils: the pilot field tests revealed a better growth of the spontaneous flora in soils that are very eroded by atmospheric agents and with a low quantity of agricultural soil (backfill).

6.1 Effect of POREM bioactivator application: field tests in Italy and Czech Republic

In order to investigate POREM activity under field conditions on several soils, pilot field tests were carried out in Northern and Southern Italy both on vegetable and arable crops as well as in Czech Republic on cereals. The results showed significant fertility improvement: the needed mineral fertilizer decreases and the yield and crops quality grows. These field tests were focused on the soil restoration to reduce degradation.

- A first pilot test carried out in 2019-2020 on a conventional production of **industrial tomato** and of **head cabbage** in two-years rotation over the same land area in Emilia-Romagna region (Northern Italy). The assessments were carried out on two fertilisation treatments: POREM with **40 nitrogen units/ha** and chemical fertiliser (NPK 26-46-50) with **130 nitrogen units/ha**, used as reference standard; an untreated check was also included for the comparison of results.
- In a second pilot test carried out carried out in 2019-2020 in Puglia region (Southern Italy), POREM was tested on organic **barley** production in rotation with **horse bean** production. Similarly, to the test on vegetables, the assessments were carried out on two fertiliser treatments (POREM with **80 nitrogen units/ha** and an organic fertiliser BIOAZOTO N12 with **80 nitrogen units/ha** used as reference standard); an untreated check was set over a similar area for comparison during the assessment tasks.
- In a third pilot test conducted in Czech Republic, POREM was tested on winter **wheat**. The assessments were carried out on three increasing doses of POREM: **117, 175 and 350 nitrogen units/ha;** an untreated check was set over a similar area for comparison during the assessment tasks.

Fig. 11 POREM on tomato production in Italy (source: 2019 pilot test in Emilia-Romagna Region – ASTRA)

Fig. 12 POREM on cabbage production in Italy (source: 2020 pilot test in Emilia-Romagna Region – ASTRA)

Fig. 13 POREM on barley production in Italy (source: 2019 pilot test in Apulia Region – ASTRA)

Fig. 14 POREM on wheat production in Czech Republic (source: 2020 pilot test in Mistřice – EPS)

Vegetable and arable tests results

For the test on tomato and cabbage crops (vegetables) in Emilia-Romagna, POREM treatment showed a significant better performance, in terms of marketable fruits, NDVI, vigor, fruit production and °Brix parameter in comparison with the untreated check, and even superior than the standard fertiliser for °Brix index.

For the test on barley and horse bean (arable crops), in Puglia region, POREM treatment showed a significant better performance in terms of ground coverage, vigour, absence of foliage yellowing (severity and incidence), plant and production improvement, in comparison with the standard fertilizer and the untreated check. POREM give a certain “visual starter effect” on barley crop in all the parameters assessed. Nitrogen provided by POREM looks more efficient in the early crop stage that nitrogen provided by standard fertilizer, due the fact that better results were obtained with the same quantity of available nitrogen.

The results assessed suggest that the bioremediation effect provided by POREM is more evident in ‘poor’ and degraded soils. Based on these considerations, POREM is an innovative material proposed as a sustainable fertilizer for degraded land. The development and use of POREM provides also an increase in the environmental friendly and sustainable production.

6.2 Effect of POREM bioactivator on degraded land and almond: field tests in Spain

Two field tests with POREM bioactivator application to soil were performed in order to obtain agricultural land rehabilitation on a typical degraded area of South-East Spain, in Santomera Murcia (started in 2019 up to 2021):

- A first pilot test was carried out on a land without vegetation, with the aim of observing the natural plants development in conditions of presence or absence of POREM in a degraded land.
- A second pilot test was carried out in order to investigate the POREM as soil organic conditioner for planting almond trees. Almond is considered a reference tree for semi-arid areas.

On both tests, the assessments were carried out on two increasing doses of POREM of 125 tons/ha each, that means in a single application over one year or repeated over two years on the same area. On untreated check without any fertilizer added was used for comparisons.

Therefore, the main objective of multi-years tests conducted in Spain was to investigate the activity of repeated applications of POREM over several years in comparison to a unique application in a single year.

The tests were located in an arid-/semi-arid land that represented a typical degraded soil for the region, with loss of natural plant cover and loss of soil quality, in abandoned areas where the change in its use is a growing problem.

Fig. 15 Degraded land in Spain, Santomera, Murcia (source: 2020 pilot test - MICRONADIR)

Non-crop and crop tree tests results

In the first crop-free test, natural spontaneous vegetation appeared, showing significant difference in plant cover respect to non POREM application. These plants act, once their vegetation cycle was finished, as new organic matter source: the process consists in reactivating the soil biogeochemical cycles that were maintained in the following years through new vegetation appears in Spring which residues go back to the soil in Summer; fact that it did not occur in the plots with no POREM addition.

In the second test, were POREM was applied as soil organic conditioner before almond tree plantation, it was demonstrated how the application of POREM produced higher vigour plants than in the untreated control. In general, the treated almond trees increased the trunk diameter in 6 mm in the first two months in comparison with the untreated plants, further the leave biomass was multiplied by three in comparison with control. The addition of POREM permitted a better root establishment that it was represented with the above-mentioned vigor. It is well documented that the initial root establishment is crucial for the future plant development, therefore it can be stated that addition of POREM is an adequate strategy for revegetation of almond trees.

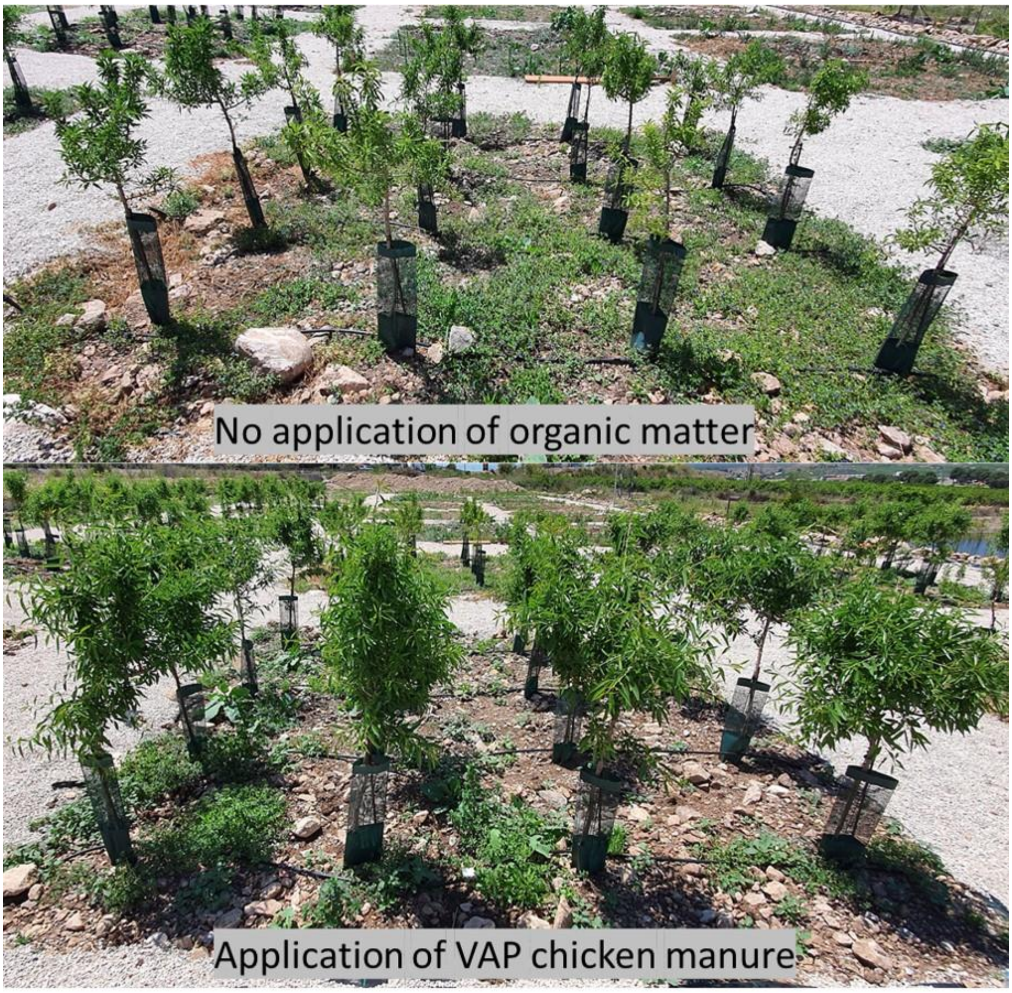


Fig.16 POREM on almond plantings in Spain (source: 2021 pilot test in Santomera, Murcia – MICRONADIR)

6.3 Conclusions

The pilot campaign of POREM production and its application yielded some important results. The POREM bioactivator is obtained from a natural treatment of the bio-product poultry manure i.e. poultry industry waste, thus adhering to the practices of the circular economy. The physico-chemical characterization has shown the time evolution of POREM bioactivator properties over time, which tend to stabilize at around 60 days; the mineralization also is time-dependent: it grows over that time span and the mineral compounds arising from it, which are related to nutrients, could become a basin of N or P for soils. Moreover, the detected growth of bio-activator stability related to the maturation time is a first demonstration that the simplified and static process of POREM production can be consider valid. The replicability of POREM properties at pile and sample is related to POREM process replicability and can be contemplated as another demonstration of POREM process efficacy. The detected intrinsic properties of POREM bioactivator, that is a natural product, rich in C and micronutrients, make it useful to soil and plants.

Indeed, several multi-year field tests performed in Italy, Czech Republic and Spain have highlighted the bioremediation effect provided by repeated applications of bioremediation fertilizer POREM on soil restoration in different European environments: POREM showed an improvement of biofertilization effect in conditions of different crop rotations (vegetables and arable crops) as well as on tree crops and on crop-free degraded soils. A general important result using POREM in the degraded lands, consists in a positive effect improving the nutritional aspect, functionality, and fertility including organic matter since the first year of application. In addition, from these tests it was demonstrated that POREM obtained a certain biostimulant effect (according to the Germination index test), and dilution tests demonstrated that the potential negative effect from salinity on seed germination is avoided. At the beginning of crop development, the bioremediation effect is more evident especially in "poor" and degraded soils, as demonstrated by the detected "visual starter effect": nitrogen provided by POREM appears to be more efficient in the early crop stage than nitrogen provided by standard fertiliser. The struvite presence accounts for a N basin which becomes available.

Finally, the field pilot tests carried out in Italy and Czech Republic showed positive results in terms of crop growth and of quantity and quality of the harvested product obtained after repeated applications of POREM bioactivator to the soil. A similar result was confirmed in Spain, were the land that received a second year of POREM bioactivator application obtained enforced positive parameters in comparison to one single year application or untreated land.

The summary table below shows the parameters observed in the tests as significantly different in comparison to the untreated check, after the POREM application in degraded soils of Italy, Czech Republic and Spain.

Table 2. Efficiency of POREM bioactivator (significant results)

VEGETABLES: *TOMATO & CABBAGE*	**CEREALS:** *BARLEY & WHEAT*
POREM *vs* Untreated ↑ Increase marketable product ↑ Increase NDVI ↑ Increase °Brix index (tomato) ↑ Increase head size grading (cabbage) **POREM *vs* Standard fertilizer** ↑ Increase °Brix index (tomato) ↑ Increase vigor (cabbage)	**POREM *vs* Untreated** ↑ Increase ground coverage ↑ Increase vigor ↑ Absence of foliage yellowing **POREM *vs* Standard fertilizer** ↑ Increase Ground coverage (barley)

NATURAL VEGETATION CROP-FREE LAND: *DEGRADED SOIL REHABILITATION*	**ALMOND TREE:** *NEW TRANSPLANTATION IN DEGRADED SOILS*
POREM *vs* Untreated ↑ Natural vegetation after 1 application over one year ↑ Natural vegetation after 2 applications over 2 years ↑ Reduce of soil degradation ↑ Reactivate of biogeochemical soil cycles ↑ Increase soil organic matter	**POREM *vs* Untreated** ↑ Increase vigor ↑ Increase trunk diameter ↑ Increase foliar biomass ↑ Reduce soil degradation ↑ Increase natural fertilization ↑ Increase biological fertilization

7. LCA

The Poultry Manure Based Bioactivator for Better Soil Management Through Bioremediation (POREM) project aims at demonstrating the restoration capacity of repeated applications of poultry manure (properly treated) to soils located in semi-arid climate zones. Its replicability in exploited soils is guaranteed by providing technical training and supporting tools for the case-to-case identification and implementation of the best technique and by using all media available to actively transfer knowledge at all levels, local, national and European. The Life Cycle Assessment is the methodology used to provide information concerning impacts related to POREM production and to compare POREM with similar commercial products (i.e fertilizers).

POREM project wants to achieve better soil management (decreasing erosion, maintaining soil organic matter, avoiding compaction and contamination, conserving/restoring carbon rich soils, etc.) at a local, regional or national level. The methodologies applied include monitoring tools and practices or the improvement of administrative and legal frameworks.

The goal of the LCA study is twofold:

- To assess the environmental profile of the production of POREM project;
- To compare POREM product with similar commercial products.

The study is commissioned by the POREM project partners and it is intended to support the quantification of some of the expected environmental benefits declared in the project.

The target audience is technical, internal and it is represented by the project partners and the LIFE Project Officer.

The study is not intended to support any comparative assertion to be disclosed to the public.

POREM is a sustainable fertilizer for degraded soils with bio-activation properties based on natural resources: it is obtained from poultry manure, poultry litter and dejections (PM in the following) treated with a natural enzyme preparation derived from vegetable active principles (VAP) and represents a continuous source of organic matter and nutrients for soil restoration.

The process of POREM production consists in 90 days cycle during which the PM, stowed in heaps, is inoculated with the VAP enzymes triggering the stabilization of the material; bio-activator stability is correlated with maturation time. The PM piles must be protected from atmospheric events, i.e. set in an agricultural barn or shed, neither moved nor mixed during the whole period.

During the maturation process, in the PM matrix, thanks to the effect of VAP, a microbial flora that is able to stabilize the different nutritional elements present is selected.

The functional unit of the study is **1 kg of POREM product**.

The study is from cradle to gate, i.e. from the production of raw materials until the production of POREM. The use phase is not considered in this study because it is treated in other work packages of this project.

A comparison was made using as parameter the Nitrogen content of the products, analysis prove that the content of N is 1 kg per 37 kg of product. Two different NPK fertilizers have been chosen to be compared with POREM, a standard NPK (15-15-15) fertilizer from Ecoinvent database and another one modelled starting from Nitrogen, Phosphate and Potassium fertilizers whose composition can be found in LCA report.

Table 3 Comparison between POREM and NPK fertilizers

Impact category	Unit	POREM as N	NPK (0,4 N)	NPK (15-15-15)
Climate change	kg CO2 eq	5,34E-01	8,82E+00	7,06E+00
Ozone depletion	kg CFC11 eq	6,61E-08	6,38E-07	8,71E-07
Ionising radiation	kBq U-235 eq	2,35E-02	4,47E-01	6,10E-01
Photochemical ozone formation	kg NMVOC eq	3,07E-03	3,71E-02	1,64E-02
Particulate matter	disease inc.	4,25E-08	3,05E-06	2,86E-07
Human toxicity, non-cancer	CTUh	5,93E-09	1,14E-07	1,23E-07
Human toxicity, cancer	CTUh	3,22E-10	7,62E-09	4,01E-09
Acidification	mol H+ eq	4,32E-03	4,47E-01	4,50E-02
Eutrophication, freshwater	kg P eq	5,03E-05	2,76E-03	1,62E-03
Eutrophication, marine	kg N eq	1,78E-03	3,25E-01	6,43E-03
Eutrophication, terrestrial	mol N eq	1,89E-02	1,85E+00	1,56E-01
Ecotoxicity, freshwater	CTUe	6,67E+00	3,22E+02	2,21E+03
Land use	Pt	1,57E+01	5,23E+01	4,96E+01
Water use	m3 depriv.	2,26E-01	4,72E+00	2,79E+00

Resource use, fossils	MJ	5,08E+00	9,04E+01	1,02E+02
Resource use, minerals and metals	kg Sb eq	9,57E-07	2,31E-04	1,37E-04
Climate change - Fossil	kg CO2 eq	3,65E-01	8,80E+00	7,04E+00
Climate change - Biogenic	kg CO2 eq	1,19E-01	9,63E-03	7,53E-03
Climate change - Land use and LU change	kg CO2 eq	4,93E-02	1,31E-02	1,68E-02

Comparing POREM to NPK fertilizers with the same nitrogen content, results show how POREM has better performance from an environmental point of view for almost all the impact categories analysed. POREM appears to have worse performance only for land use indicators that are related to the production of plant used in the production of enzymes for the activation of POREM. In addition, after the normalization phase, these indicators were found to be not significant.

Considering for example Climate Change (Figure 17 and 18Figure), POREM has very lower emissions in the comparison with NPK fertilizers. If we look at the contribution of single process to the total Climate Change indicator, we can see how impacts are due to transportation of poultry manure, the production of milk used for VAP fabrication, and the farm tractor used to produce POREM.

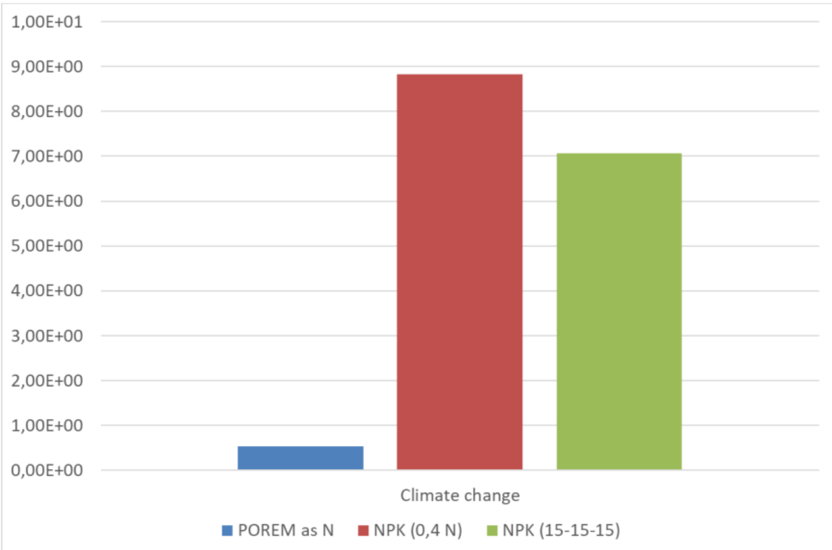


Figure 17 Comparison between POREM and NPK – Climate Change

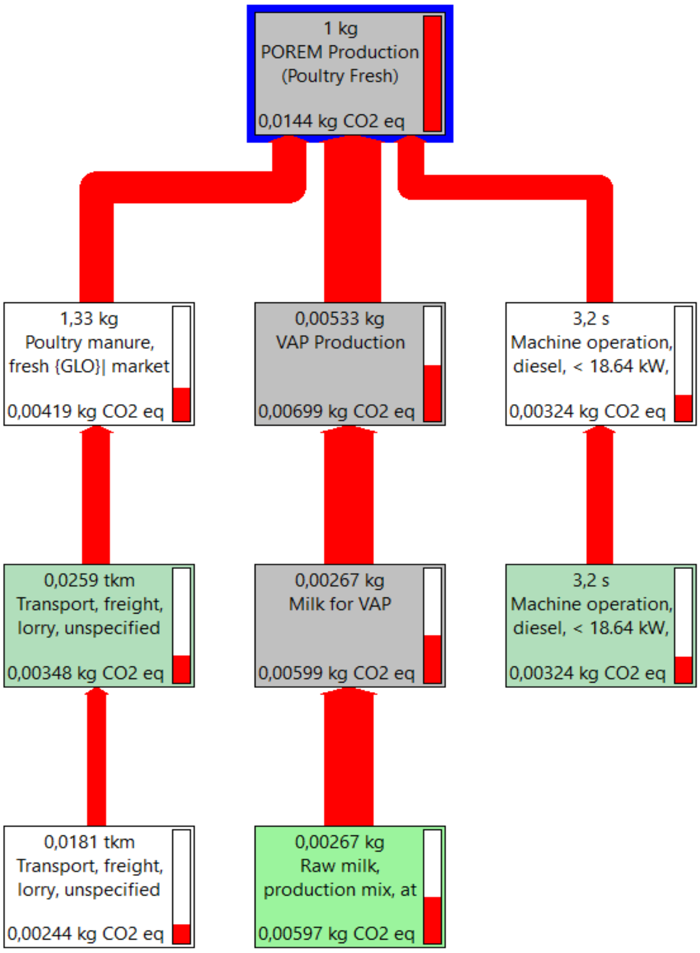


Figure 18 Contribution of the process for the POREM production to the impact category Climate Change (kg CO_2 eq)

The production of POREM is a simplified process which provides a clear example of the concepts of Circular Economy. The main component, poultry manure, is usually considered as a waste/by-product with all the difficulties related to its management, in fact, to date, only a part can be reused as fertilizer after a pre-treatment phase; to be able to treat the remaining part, a final site must be found, this occurs through the spreading of poultry manure on dedicated soils that are not always available, in fact, definite limits cannot be exceeded to prevent phyto-toxicity effects due to excessive distribution on soils.

Through the activation of the poultry manure thanks to the use of enzymes (VAP), POREM acquires characteristics similar to an NPK fertilizer but with some extra qualities that characterize it as a bio activator rather than a fertilizer, for example, POREM has the ability to release nutrients more slowly than a chemical fertilizer.

Analysing the impacts related to the production of POREM we can see how in the comparison with two types of NPK chemical fertilizers, equals in their N content, all the impact categories analysed have lower values. If we analyse the impacts due to POREM production, we can see that the process is already quite optimized, an advantage could be obtained by optimizing the logistics.

Producing POREM directly at the production site could avoid transportation and thus eliminate the associated impacts.

The analysis focuses on the production phase, but the advantages can be more significant if we consider the potential benefits due to the recovery of marginal soils. In fact, as seen during the project, advantages that may derive from the use of POREM compared to the use of a normal chemical fertilizer, are much greater, from field tests we have seen how POREM has the ability to recover marginal soils (increasing the content of carbon and nutrients) for agricultural purposes.

8. LEGISLATIVE FRAMEWORK

8.1 Regulatory framework

The product impacts with different sectors. For example:

- **Legislation related to Animal By-Products (ABP)**

  - Regulation (EC) No 1069/2009 of The European Parliament and Of The Council of 21 October 2009 Laying down health rules as regards animal by-products and derived products not intended for human consumption 56 articles and 1 attachment) and subsequent amendments. The regulation is of interest to the sector as it governs the placing on the market and use of fertilizers based on animal by-products.

  - Regulation (EU) No 142/2011 of 25 February 2011 establishes the application of EC Regulation 1069/2009 animal by-products.

- **Legislation related to fertilizers**

  - Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019, laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003.

  - Legislative Decree 75/2010 of 29 April 2010, concerning the placing of fertilizers on the market regulates biostimulants.

- **Legislation related to nitrate emissions**

  - Council Directive (EU Nitrates Directive) 91/676/EEC of 12 December 1991, concerning the protection of waters against pollution caused by nitrates from agricultural sources[5].

  - Regional Decree No 209 (regulation No 3) of 15 December 2017 (Regolamento regionale in materia di utilizzazione agronomica degli effluenti di allevamento, del digestato e delle acque reflue) of Emilia-Romagna region, concerning the agronomic use of livestock manure, digestate and wastewater. The regulation also establishes that the amount of total Nitrogen to the field brought by livestock manure must not exceed 340 kg/ha per year. Each Italian region has its own regulation of the digestate issue (Emilia-Romagna, Italy).

- **Legislation related to organic agriculture**

  - Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.

  - Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, fertilizers labelling and control.

  - Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007.

[5] Livestock manure Definition "waste products excreted by livestock or a mixture of litter and waste products excreted by livestock, even in processed form"

- Ministerial Decree 6793/2018, Provision for the implementation of EC Reg.s No 834/2007, No 834/2007, No 889/2008, No 1235/2008 and subsequent amendments regarding organic production and labelling of organic products (Italy).

8.2 European policies targeted

On the basis of obtained results, the POREM project can give a contribution to interdependent agricultural/environmental policies.

- Soil degradation is an important issue of **Thematic Strategy for Soil Protection** (COM (2006)231). LIFE POREM objectives are fully consistent with the European Thematic Strategy for Soil Protection addresses. Demonstrative tests carried out during the project showed the process inversion of organic matter loss in soil, thus preserving its functions. The POREM bioactivator applications will prove crucial for both the restoration of damaged soils and the prevention (by means of an effective monitoring) of future degradation.
- The project is in tune with European environmental policy and legislation, since soil depletion and its consequences have both biophysical and socio-economic dimensions and they affect agricultural and marginal land. **Common Agricultural Policy** has progressively included more environmental requirements. Another example is: appropriate soil management is essential for many Natura 2000 sites, and the limiting impacts of marginalization will also be relevant in meeting the requirements of the Birds and Habitats Directives.
- Soil restoration is obtained by bioactivators based on the use an animal by-product (poultry manure specifically), thus matching European policies of **Circular Economy Action Plan** (CEAP), Com/2019/190, transforming a by-product into a new product with resource saving (recycled nutrients, particularly P), industrial Symbiosis network.
- POREM contemplates some specific problems, which are interesting to highlight: 1) The need to increase the fertility of our agricultural soils, in order to achieve efficient and sustainable production systems; if we are not able to conserve the soil, agriculture will not be able to feed the entire existing population within a few years; 2) The need to reduce inputs for agriculture, and therefore, to promote the contribution of nutrients through organic amendments, which contribute to the fertilizing needs of crops, in addition to generating a soil with great added value: biodiversity, functionality and sustainability; 3) The need to walk towards an agriculture that is more in line with the environment, that avoids the massive use of inputs and that does not contribute to generating problems that increase climate change.
- From an agronomic point of view, POREM application allows to reduce the quantity of mineral fertilizer used in the crop production in a significant quantity (in the tomato test this reduction was near 70% in available Nitrogen). This fact will benefit first the farmer, that will improve the sustainability of their crops (more suitable for consumers sensitive to the ethical contents of food) and the productivity on long terms of their soil (less risk of salinization and increase of organic matter). The citizen will benefit the reduction in fertilizer by the decrease of leaching Nitrogen in water table (drinkable water).
- The project results which show the potentiality to englobe ammonia in struvite in bioactivator, with low water solubility, can reduce the potential for water pollution, particularly for ground and surface water (Nitrate directive CEE/91/676).

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