International Journal of Agriculture Innovations and Research
Volume 7, Issue 5, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 25/02/2019 | Accepted on: 19/04/2019 | Published: 05/05/2019

Reduced Salinity Fertilizer from Compost, Manure and Biochar (at Lab Scale)

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Abstract – **"Turning waste into a resource" is a key action of circular economy policy. This paper deals with the production of reduced salinity fertilizers (RESAFE) for chemical and mineral fertilizers integration/ substitution through a technological route based on the processing of urban organic waste (UOW), farm organic residue (FOR) in presence or not of bio-char. The work was carried out within the framework of the RESAFE LIFE project named "Innovative fertilizer from urban waste, bio-char and farm residue as substitute of chemicals fertilizers" in which innovative oxidative/ composting strategies and monitoring techniques were utilized to obtain High Quality Organic Fertilizer1. The goal was to demonstrate an innovative biotreatment and monitoring technique to obtain RESAFE. Both the process and fertilizer final quality were monitored using chemical and physical parameters (pH, dry matter, salinity, TOC, TN, Csoluble, Nsoluble, P, K, micronutrients), enzymatic activities, basal respiration and mineralogical phases via X-Ray Diffraction (XRD). Also morphological aspects via SEM characterization were observed. The results of lab scale tests are presented, in order to select final recipe to be used in pilot plant and subsequent agronomic field tests.**

Keywords – **Biochar, Enzymatic Treatment, Fertilizer, Poultry Manure, Struvite, Urban Organic Waste.**

I. INTRODUCTION

Population growth leads to an increase in demand for food and energy, and therefore for crop production and productivity. Fertilization is one of the pillars of crop production [1], together with the maintenance of soil health and fertility. The fertilizers need and production are growing: FAO estimates world consumption of the three main fertilizer nutrients, nitrogen (N), phosphorus expressed as phosphate (P2O5) and potassium expressed as potash (K2O) in 186.67 million tons in 2016 and the demand for N, P2O5 and K2O is forecast to grow annually on average by 1.5, 2.2 and 2.4 percent respectively from 2015 to 2020 [2].

The first approach to increase crop production and productivity is directed at improving the use efficiency of existing fertilizers, through better management practices: 4R principles of nutrient stewardship (right source/ fertilizer, right rate, right time and right place [1]).

The second approach is the development of always best performing fertilizers, which is the goal of many companies and researchers. In this case, improving efficiency, the same productivity is obtained with lower amount of fertilizers, combined with their reduced loss towards environment (water and soil).

Many strategies to improve efficiency have been applied and investigated, only concerning fertilization. The employ of slow release fertilizers or controlled-release fertilizers is a first solution for N and P fertilizers. This solution allows nutrient to be more available along time for vegetable and to minimize losses to leaching, runoff or to the atmosphere. It can be obtained: i) disrupting soil reactions that enhance mobility, i.e. using urease or nitrification inhibitors; ii) coating a soluble nutrient source to delay or control release (coated fertilizers, such as polymer coated fertilizers or NCU – neem coated urea); iii) using a less soluble nutrient source (i.e. IBDU- isobutyldenediurea, struvite for N and P or langbeinite for K) [1], [3]. Recovering phosphorus from waste streams as struvite is an example of fertilizer with delayed nutrient release, proper for special crops.

Other strategies for productivity increase are related to the integration of major nutrients (N, P, K) with the desired concentration of micronutrients (micronutrient containing fertilizers). In fact, the lack of micronutrients (copper, zinc, etc.) reduces the performances also of major nutrients (N, P) [1].

According to these scenarios, mineral fertilization is complemented by the use of products from waste streams and livestock residues.

Historically, treatment of bio-waste and organic fraction of urban wastes, from separated collection, have been developed and optimized in order to obtain organic products (compost) to be used as soil amendment when integrated with nutrients/ fertilizers for crop harvesting, with the aim at improving soil fertility as far as physical, chemical and biological properties. This method allows a proper final disposal for wastes, giving back to soils part of the organic matter they produced. Carbon is returned to the soil (sink), thus reducing the Greenhouse Gas emissions (GHGE), even if from organic and renewable sources. Furthermore, the contribution of organic matter to landfills is reduced, thus decreasing CH4 emissions, the greatest contributor to the GHGE effect in landfills. For example, landfills are the third largest source of CH4 emissions in the United States [4].

On the other hand, a large amount of livestock residues is produced, particularly manure. Very often their production is concentrated in restricted areas, and there are problems for their final disposal in surrounding farmland. They are a large source of organic carbon, major nutrients (N and P, [5]) and micronutrients, with different properties and composition in function of the kind of animal and the rearing methods, but also of gaseous emissions of ammonia (NH3), methane (CH4) and GHG [6]. Furthermore, an improper direct use may cause problems of phytotoxicity and damage to soils, because of salinity as well. Salinity is closely linked to soil degradation issues, including soil erosion. It is often associated with prolonged wetness and lack of surface cover and, therefore, increases the vulnerability of soils to erosion.

Other kinds of carbonaceous by-products are now available as Biochar (BC), a solid product created by pyrolysis of biomass that can be used to lock carbon in the soil: BC, in fact, is very stable since is not accessible to normal microbial decay, turning the soil into an actual carbon sink [7]. It is mainly composed of aromatic compounds, that are not organized in adherent layers, depending both on pyrolysis temperature and the kind of biomass used as feedstock [8].

In connection with waste management, composting and pyrolysis with biochar production are methods to reduce GHGE [9], since C sequestration in soil is recognized as a mitigation measure of GHGE by IPPC [10]. Additionally, BC can prevent the leaching of nutrients out of the soil, partly because it absorbs and immobilizes certain amounts of nutrients [11]. Modest additions to soil were found to reduce N2O emissions by 30% [12] and can influence also methane emissions [13].

The use of agricultural char is ancient and documented [14]. Important BC properties are specific surface area and pore volume: their very large porosity consists of pores with a wide range of pore size, from macro to micro porosity. Macro pores (pore size > 50 nm) play a key role in water retention and help plant roots gain access to water and nutrients [15], [16]. The micro porosity (pore size <2 nm) is the consequence of the pyrolysis condition (temperature, dwelling time, etc.); this porosity greatly influences the surface area and is mainly responsible for high BC absorption capacity. On the negative side, if BC has a high C/N ratio, it may result in net N immobilization, thus its potential use as soil amendment is pre-empted [17]. Soils require active carbon to maintain micro and macro populations and a correct C/N to prevent N immobilization.

The organic fertilization becomes essential in order to increase the organic matter content in the soil after incorporation. Soil organic matter plays an important role in soil structure and contributes to many of the reactions that take place, such as water penetration, root development, etc. [18]. It is also the storage sphere for the major nutrients, such as nitrogen, phosphorus, Sulphur and micro-nutrients.

The work described in this paper, carried out within the framework of the European RESAFE LIFE12 ENV/IT/356 project (Innovative fertilizer from urban waste, bio-char and farm residues as substitute of chemicals fertilizers), is therefore targeted to obtain high quality organic Reduced Salinity Fertilizers (RESAFE) from innovative composting strategies [19], [20]. Raw materials were selected in the categories UOW-FOR-BC (Urban Organic Waste, Farm Organic Residues and Bio-Char).

The foreseen advantages of RESAFE fertilizers are:

- A reduction in systematic utilization of chemical fertilizers, up to 50% (project objective);
- an improvement of soil health and fertility via an increase of organic matter in the soil, which can reduce soil tiredness, improve the porosity of the soil, reduce compaction, improve the drainage and the aeration of the roots and, finally, can strongly contribute to enhance the absorption of mineral elements;
- An increased availability of N and P for the plants, connected to a higher content of organic matter and nutrient compounds.

The aim of the project was to investigate at laboratory level the contribution of BC for the production of Reduced Salinity Fertilizers (RESAFE) derived from biowaste and animal manure. The properties as fertilizer of products have been monitored in order to define a proper recipe for pilot plant and then use in partial substitution of mineral fertilizers. In particular evolution of Nitrogen availability during preparation, bio stabilization via enzymatic activity and respiration have been monitored together with the compresence of micro-nutrients. Microbiological and biochemical parameters but also morphological and mineralogical characterization have been carried out. A simplified, enzyme aided, bio treatment was applied.

II. RESAFE PRODUCTION

A. *Raw Material Selection*

Many different types of organic waste have been recently regarded as original raw materials for compost. However, the basic chemical structures in all those materials are largely similar, and basically comprised of three carbonaceous constituents: cellulose, hemicellulose, and lignin. RESAFE was built on the basis of organic waste & by-products, considering the following features:

- Organic matter (OM) content and stability;
- Nutrient content and salinity;
- Biological activity for C, N and P cycle;
- Local availability, for the purpose of improving recycle of local organic waste and by-products.

Composts produced from separated collection of UOW, were selected in order to create a base of semi-labile OM.

Among FOR, poultry manure was selected because of its plenty of nutrients (particularly N and P) and local availability. Manure from intensive laying hen farm was used, after drying by external tunnel, recognized as BAT [21].

BC samples were selected from lignocellulosic raw material pyrolysis. BC with a specific surface area of 22.2 m^2/g (BET method) and a porosity greater than 80% was used. The pore size distribution showed a large amount of pore clustered between 1 and 3 µm.

The characteristics of the selected raw materials are summarized in Table 1. Data are expressed as mean value of 3 sample and standard deviation.

Table 1. Raw materials characteristics.

		UOW	BC	FOR
pH (Dil 1/10)		8,31	10,79	7,29
EC (Dil 1/10)	mS/cm	2,89	2,70	6,57
Total C	(g/100g)	17,90	30,80	33,13
	σ	0,76	0,20	0,82
Organic C	(g/100g)	15,25	29,80	32,11
	σ	1,12	0,68	0,69
Soluble C	%	0,73	0,60	4,95
	σ	0,05	0,01	0,10
Total N	(g/100g)	1,49	3,80	4,95
	σ	0,11	0,05	0,27
Soluble N	%	0,18	1,00	1,22
	σ	0,01	0,01	0,03
b-Glucosidase	µmol PNF/g h	7,31	0,00	9,44
	σ	0,25	0,00	0,23
Phosfatase	µmol INTF/g h	56,52	0,00	201,90
	σ	3,53	0,00	4,74
Accumulative Respiration	mg C-CO2/kg d	7230	2813	110642

B. *Raw material treatment*

A simplified energy saving biotreatment was provided in the project for the selected raw material. The biotreatment consisted in adding the substrate with a natural enzyme preparation, Vegetable Active Principles (VAP), in a batch static process according to an EU patent [22]. The VAP preparation used a mixture of herbaceous plants belonging to the Cucurbitaceae, Graminaceae, Labiatae, Apiaceae and Rutaceae families or parts thereof.

The natural, enzymatic biotreatment had already been tested in different applications:

- In biostabilization tests of undifferentiated UOW organic fraction from mechanical sorting with "non-conventional" technologies. In that case, biotreatment was aimed to speed up the waste stabilization process; moreover, best results were obtained by merely adding VAP preparation enzyme cocktails to a static process, without any aeration and/or periodic turning [23];
- in poultry manure biostabilization and transformation into an organic fertilizer characterized by slow release nitrogen [24];
- In the deodorization and conversion of poultry manure into an innovative ecofriendly bating agent for leather tanning [25].

In this work, the biotreatment was applied to stabilize the mixture of organic matrices, to reduce salinity in order to obtain a high-quality organic fertilizer, rich in micro and macro nutrients, capable to substitute/reduce mineral fertilization.

C. *Lab Scale Demonstration Tests*

Based on raw materials characterization, two recipes were tested:

a) A mixture of compost (UOW) and dried laying hen manure (FOR) was used as feedstock for the tests (50: 50, w: w, wet weight). Compost, in fact, is more stable than FOR, but FOR is very rich in nutrients. The aim of the high FOR percentage is to obtain a final fertilizer which can effectively replace mineral fertilizers. In addition, FOR showed higher figures for organic carbon content and enzymatic activities.

b) 10% Biochar (BC) was introduced in the previous mixture, according to other works [14], as a very stable organic material that can improve the final product. The initial mixture UOW + FOR + BC was provided for at the following rate (45% - 45% - 10%) to guarantee a good structure and moisture.

The two recipes were subjected to biotreatment with VAP, in order to obtain respectively UOW + FOR + VAP (UFV) and UOW + FOR + BC + VAP (UFVB) fertilizers (Fig. 1).

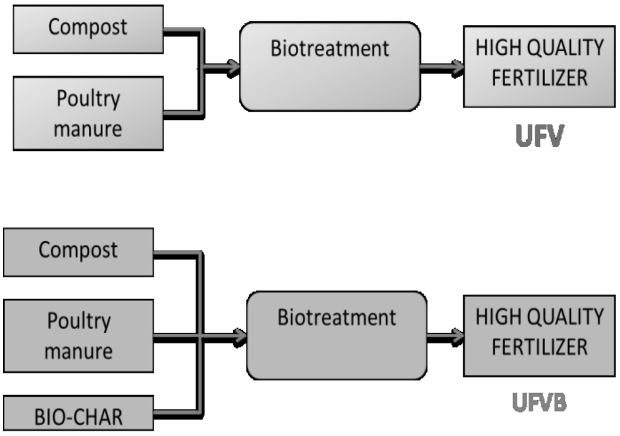

Fig. 1. Diagram of RESAFE production processes.

Bioreactors of approx. 75 L with cover (cap) were chosen for the biotreatment (Fig. 2), and three replicas for each recipe were produced. The biomixes for UFV and UFVB with the correct proportion were obtained with manual mixing and then distributed to the 3 bioreactors simultaneously. A layer of biomix was laid on the bottom, the VAP preparation was added and then covered with the remaining part of biomix, and filled up to the top. About 3 kg of VAP were added for about 38 kg of bio-mix. Static batch ripening and bio-stabilization processes took place inside bioreactors for a time period up to 60 days.

Fig. 2. Bioreactors during filling and during maturation.

The sampling was scheduled at T0 (at the beginning of the experiment) and with a frequency of 20 days (T20, T40, T60). The initial sample was taken from the mixed pile during bioreactor filling. Three sub-samples of about 0.5 kg were taken from each recipe and bioreactor, mixed and homogenized.

III. ANALYTICAL METHODS

Different analytical techniques were adopted in order to monitor the evolution of initial biomix and to verify final fertilizer quality. In particular, the following parameters were taken into account.

- Physical/ chemical and chemical parameters as descriptors of organic matter evolution and nutrient contents. pH, salinity, moisture, ashes. Carbon: total Carbon (TC), total organic carbon (TOC), water-soluble Carbon (C_{sol}). Nitrogen: Total Nitrogen (TN) and water-soluble N(N_{sol}). Macronutrients: Phosphorus, Potassium and Magnesium.
- Microbiological and biochemical parameters: microbial respiration and enzymatic activities, such as β–glucosidase and phosphatase enzyme, as indicators of transformation connected to C and P cycles.

Moreover, the following analyses were carried out:

- Scanning Electron Microscopy and micro-analysis (SEM-EDXRS) for morphological and semi-quantitative analyses;
- X-Ray Diffraction (XRD), to recognize mineral compounds and phases within the organic matrices.

D. *Physical and Chemical Parameters*

The pH and electrical conductivity (EC) were measured in a water suspension (1:2.5 and 1:5 w/v, respectively).

Moisture measurements were carried out as weight loss upon drying, heating the sample in an oven at 105ºC for an appropriate period (at least 4 hours) and then cooled in dry atmosphere.

Total organic C and total N content were measured by Elemental Analyzer LECO C, N, S macro samples. Water-soluble C (WSC), indicative of labile C, and soluble N, were obtained from an aqueous solution (1:5 w/v), filtered (Albet 145 110) and quantified with a Shimadzu TOC-5050A analyzer. Total P, K and Mg were also quantified using a Thermo Scientific 6000 Series ICP Emission Spectrometer after acid digestion.

E. *Microbiological and Biochemical Parameters*

Microbial respiration (Fig. 3) was analyzed by placing 50 g of each sample moistened at 40–50% of its water-holding capacity (water potential: -0.055 Mpa) in hermetically sealed flasks, which were then incubated for 22/31 days at 28ºC. The CO_2 released was periodically measured (every day for the first 4 days and then weekly) using an infrared gas analyzer (Toray PG-100, Toray Engineering Co. Ltd., Japan). The data were added to give a cumulative amount of CO_2 released after 31 days of incubation. Basal soil respiration was expressed as mg CO_2–C kg^{-1} sample per day [26].

Fig. 3. Microbial respiration analysis.

Respiration is a widely used parameter to estimate microbial activity. The activity of all heterotrophic microorganisms leads to the degradation of organic matter; such decomposition has frequently been used to indicate the biological state of soils and organic materials. CO_2 emissions have been used to measure the active fraction of the microbial biomass and organic matter available during the experimental period.

Alkaline phosphomonoesterase and β-glucosidase activities in raw materials and bio-mix were determined using the methods of Tabatabai and Bremner [27] and Eivazi and Tabatabai [28] respectively. ß-glucosidase (E.C. 3.2.1.2.1.), involved in C cycle, catalyzes ß-D-glucopiranosides hydrolysis. Its activity is of great interest in organic materials that have undergone constant transformations. The presence of phosphomonoesterase activity (E.C.1.3.1.) in organic materials is important for the correct P cycle function, since this hydrolase manages to hydrolyze phosphorus organic compounds and to transform them to inorganic forms, which are more useable by microorganisms and plants.

F. *Scanning Electron Microscopy (SEM) and Micro-analysis*

Samples were observed with a FEI Inspect S Scanning Electron Microscope, coupled with EDAX Falcon Microanalysis with Germanium detector for the elemental analysis. The electron gun is a Tungsten filament and maximum resolution range between 3 nm (30 kV in high vacuum conditions, SE and BS Everhart-Thornley detector) and 12 nm (3 kV in low vacuum, GSE Large Field detector). Analysis conditions were 25 kV, high vacuum, ET detector, work distance about 10-10.5mm. The samples were dried in HERAEUS Vacutherm at $6x10^{-1}$ mbar for 30 minutes.

Also a LEO 438 VP SEM equipped with a EDS (Energy Dispersive X-Ray Spectroscopy) microanalysis Oxford Link ISIS 300 was used. The operating condition were: variable pressure (VP) in the chamber to minimize the charging effects of non-conducting samples, 20 kV, working distance of 10-12 mm for SEM observation. Samples didn't need further pretreatments. In all samples the morphology observation and semi-quantitative analysis were performed on different areas to obtain more representative and reliable results.

G. *X-Ray Diffraction (XRD)*

XRD has been employed for recognizing the crystalline phases inside the complex biological and amorphous matrix: the crystallographic determination of the phases is based on the microanalysis data. The evolution of the mix during bio stabilization at laboratory scale was also monitored, comparing receipts with and without Biochar. Before the analysis, the samples have been dried in a ventilated hoven at 60°C until constant weight, ground within an agate mortar and homogenized.

X-ray diffraction patterns (XRD) have been recorded between 10° and 70° (2Θ) using a Philips powder diffractometer with a Bragg-Brentano geometry, equipped with a copper anode operated at 40kV and 30 mA (step 0.02°, timestep 6 sec). The phase analysis has been carried out with the PC X'pert High Score software Version 2.2a (Panalytical B.V.).

IV. Results and Discussion

Raw materials used as feedstock for the tests, compost (UOW) and poultry manure (FOR), showed a good content in TOC, TN and in other macronutrients, such as P, K, Ca, Mg as well. This can give an idea of the contribution of these elements to the fertilization of soils. Data on microbial activity indicate that, except BC, the rest of organic materials can be considered as active as far microbial activity is concerned.

The combined application of SEM and microanalysis and XRD highlighted some relevant aspects useful for the identification of the best receipt for a high-quality fertilizer by means of the morphological, crystallographic and chemical features.

H. *Physical and Chemical Characterization*

The results of chemical-physical characterization of the two recipes UFV and UFVB during the 60 days treatment are described in the following. Data are expressed as mean value of 3 samples and standard deviation.

Measured pH showed a slight increase over time during the “slowly bio-oxidative” process, reaching in all cases values close to 8.5. This increase could be due also to degradation of some organic low molecular weight acids (acetic, propionic or butyric acid, for example). Slightly alkaline pH is suited to reduce mobility/ availability of some elements, such as heavy metals when the fertilizer is added to soils.

Moisture values are around 30%, just above the required threshold for organic processes.

Salinity results, expressed as Electric Conductivity, are reported in Fig. 4.

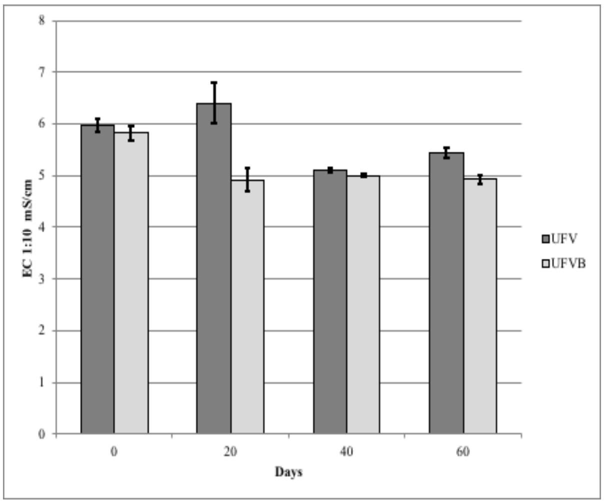

Fig. 4. Salinity.

Electrical conductivity showed high values in all initial samples, as befits high values in the starting materials, while different behaviours were detected during the ageing:

- A trend with an increase at 20 days for UFV, followed by a decrease to initial values;
- A slow continuous reduction for UFVB, with subtle, final differences compared to the initial values.

Salinity is a measure of the total soluble salts present: since its values are strongly influenced by raw materials rather than by the process, the study indicated that this parameter was not greatly affected by the different mixtures, although final values lower than the starting point were reached.

High levels of soluble salts in the root zone may affect water and nutrient uptake and adversely affect plant growth. Plants are more susceptible to salinity in their germination and seedling stages than in later stages of growth.

The recipes showed high Organic Carbon content (greater than 20%), characteristic of good amendments. For this reason, when used they will cause soil organic matter to be increased, thus improving the soil water-holding capacity and microbial activity. They also affect several critical soil functions, such as plant nutrient availability and the diversity and activity of soil organisms. The stabilization process carried out with the RESAFE experiments showed little variations for organic carbon (and organic matter) in all samples (Fig. 5). The trend, with an increase along the process, can be explained with concentration due to mass loss but also connected to substrate heterogeneity.

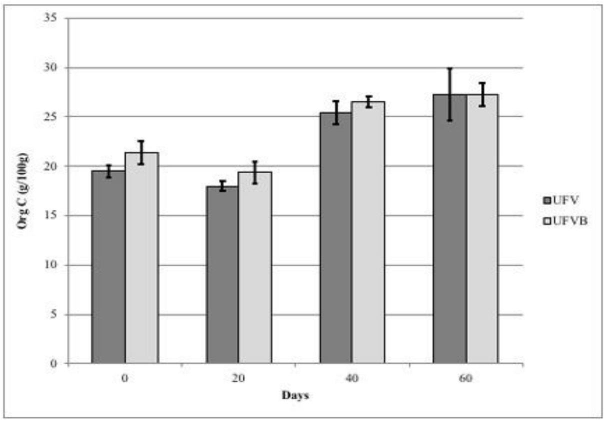

Fig. 5. Organic Carbon.

The process used is a slow, static, oxidative process, and the mineralization of Organic Carbon is not expected to be very high over a 60 days period. Total Carbon and Total Organic Carbon (TOC) are almost overlapping in value; the inorganic fraction is less than 10%, on wet basis (data not shown). As typical of these matrices, there is a very high variability. In the first 40 days, TOC seems to increase as if the CO_2 loss could be negligible.

With reference to Soluble Carbon, both UFV and UFVB have fluctuating trends but with slightly different final values, around 2.5 % (Fig. 6).

Considering that also water-soluble C compounds contribute to electric conductivity, final different salinity values could be influenced by different concentration of these C compounds. Hydro soluble Carbon is the more labile Carbon fraction; therefore, it is more easily used by soil microorganisms to obtain energy. This Carbon fraction is very dynamic, being subject to continuous processes of synthesis and degradation. The mineralization process, which takes place during decomposition, results in lower labile organic carbon, as observed by several authors. The carbon mineralization is principally due to the FOR since

BC and UOW should have a more consistent structure and are less accessible to microbial attack. The microorganisms, therefore, use the labile carbon compounds present in FOR as an energy source.

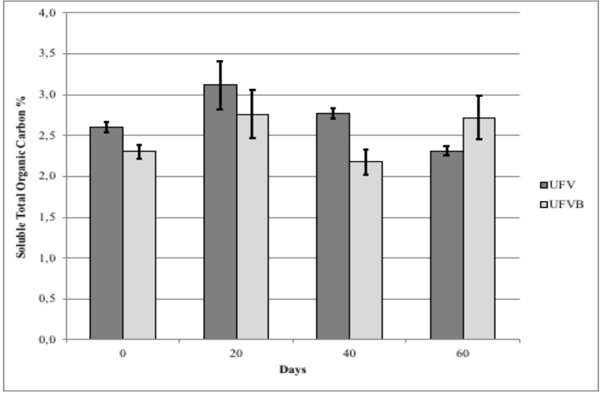

Fig. 6. Soluble Carbon.

In regard to Total N, there is a large variability in each data, connected to matrix heterogeneity as well. It is difficult to recognize trends, although average content values are around 3%, matching the target threshold for the RESAFE project (Fig. 7). TOC/TN ratio reaches the final figure of 10 for UFV e UFVB, close to figures yielded in good agricultural soils.

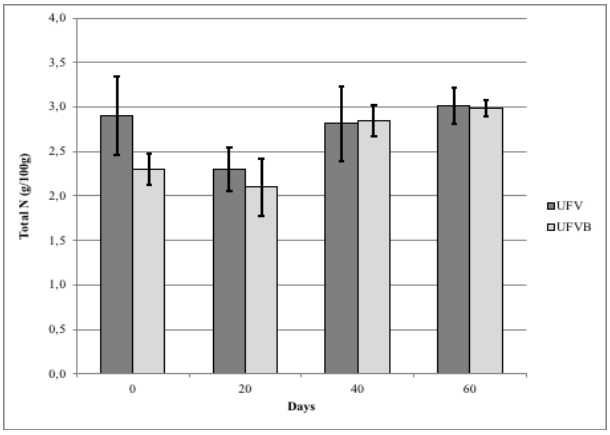

Fig. 7. Total Nitrogen

After 40 days, Total Soluble Nitrogen shows a decrease of about 30%, which can be explained by formation of compounds that are less water soluble (Fig. 8).

This reduction can be probably related to the formation of Struvite, which was detected by X Ray Diffraction analysis on pre-dried samples after 40 days (see K).

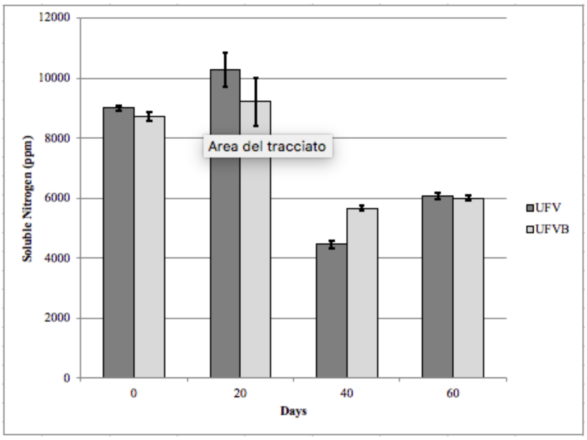

Fig. 8. Soluble Nitrogen.

The contribution of OM to fertility is well known. Within that, the water-soluble fraction is especially important [29]. The labile fraction of organic matter is the most degradable and acts as an immediate energy source for microorganisms [30]. It undergoes several transformations due to the continuous mineralization produced in organic materials, changing the contents of soluble C and N.

Macronutrients such as Phosphorous and Potassium were also measured (Table 2). Phosphorous is usually plant available as inorganic phosphate ions (HPO_4^{2-} and $H_2PO_4^-$) and sometimes as soluble organic Phosphorous. The bulk of phosphorous exists in three general groups of compounds, namely: organic Phosphorous, Calcium bound inorganic Phosphorous and Iron or Aluminium bound inorganic Phosphorous.

Most of these phosphorous groups have very low solubility and are not readily available for plant uptake. When soluble sources of Phosphorous, such as fertilizers and manures, are added to soils, they are fixed and, in time, form highly insoluble compounds which are not plant available. Fixation reactions in soils may allow only small fractions (10% to 15%) of the Phosphorous in fertilizers and manures to be taken up by plants in the year of application. Consequently, it is common to apply Phosphorous amounts two to four times the expected dose for plant uptake.

Research must focus on Phosphorous compounds with the dual properties of enhancing plant uptake (growing up the 10-15 %) and runoff prevention. Runoff is particularly an issue in cases of erosion, when soil with Phosphorous content is removed causing a Phosphorous accumulation in sediments, Phosphorous being a limiting factor for eutrophication phenomena.

Of all the essential elements, after Nitrogen and Phosphorous, Potassium is the third most likely, to limit plant productivity. For this reason, it is commonly applied to soils as fertilizer and is a component of most mixed fertilizers. Potassium is known to activate 80 different enzymes responsible for such plant and animal processes as energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation. Potassium plays a critical role in reducing the loss of water from leaves while increasing the ability of roots to take up water from the soil. A potassium content of about 2% is acceptable both for organic fertilizers and for the purposes of RESAFE project.

In order to evaluate the nutrient content of the new fertilizers, micronutrients were also detected. Table 2 reports the micronutrient as average value for final fertilizers in dry matter together with standard deviation for the two recipes, UFV and UFVB. All the examined materials contain micronutrients and trace elements (B, Ca, Cu, Mn, Zn, Na) which are very important for agricultural soils.

The final RESAFE Fertilizer quality can be connected to the presence of organic matter, macro and micronutrients, in the direction of valorising the “micronutrient-fortified fertilizer” concept [1].

Regarding the heavy metal contents measured in all organic materials, they did not exceed acceptable levels. All values fall within the ranges that can be considered "normal" for all heavy metals.

Table 2. Macro and micronutrients average concentration in 60 days UFV and UFVB samples

		UFV	**UFVB**
P	(g/100g)	1,039	0,953
	σ	0,221	0,128
K	(g/100g)	1,973	1,981
	σ	0,242	0,219
Mg	(g/100g)	0,646	0,708
	σ	0,057	0,083
Ca	(g/100g)	7,667	7,294
	σ	0,895	0,767
Na	(g/100g)	0,310	0,299
	σ	0,040	0,036
B	(mg/kg)	44,7	45,8
	σ	4,7	4,4
Cu	(mg/kg)	88,8	95,8
	σ	11,5	12,1
Mn	(mg/kg)	462,7	467,3
	σ	49,9	79,4
Zn	(mg/kg)	316,1	295,8
	σ	68,0	43,3

I. *Microbiological and Biochemical Parameters*

β-glucosidase results highlighted a common kinetics in presence of biotreatment (Fig. 9): UFV and UFVB showed an initial stability and then a sharp decline from 20 to 40 days, reaching final values of about 2.0 µmol PNF g-1 dry soil h-1, representing 25% of the initial value. Final values for all recipes underline a stable product, suitable for use in agriculture, constituting a gradually available sink.

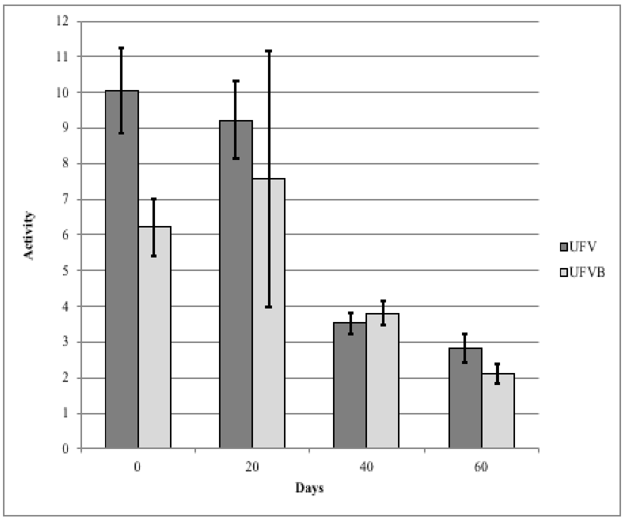

Fig. 9. β-glucosidase trend.

Phosphatase results show different kinetics (Fig. 10), which are comparable to β-glucosidase behaviour: in UFV it maintains stability for the initial 20 days, then there is a significant change between 20 and 40 days; in UFVB, the initial BC presence acts as activity inhibitor between 0 and 20 days, then it gradually it performs as the other samples.

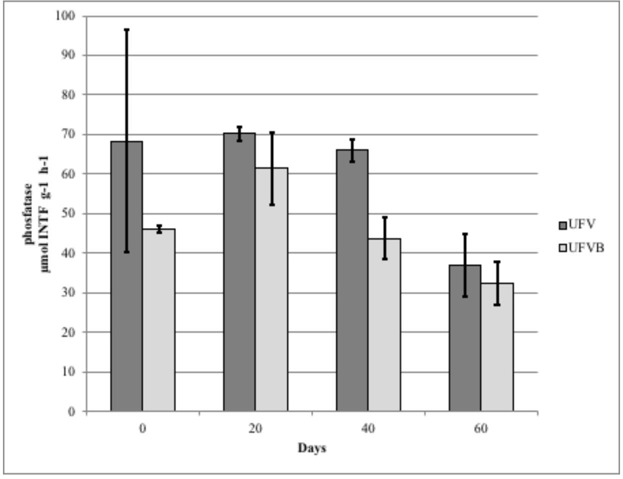

Fig. 10. Phosphatase trend.

Microbial respiration is a parameter of great interest, since it evaluates the process of Carbon mineralization and may give an idea of the amount of Carbon that can be fixed in the organic structures. The organic materials have higher respiration values, because of their incorporated organic matter. Both specific and accumulative respiration were measured. Responses for accumulative respiration are reported in Fig. 11, expressed as C-CO_2 kg^{-1}; the specific respiration trend is not reported, because consistent with the accumulative figure. For all samples, there is a first phase with a slowdown of microbial activity (20 days) while later phases show a rise in respiration. According to experience, the different phases may be due to a degradation of initial labile substrates, which is then followed by new colonization (microorganisms and fungi).

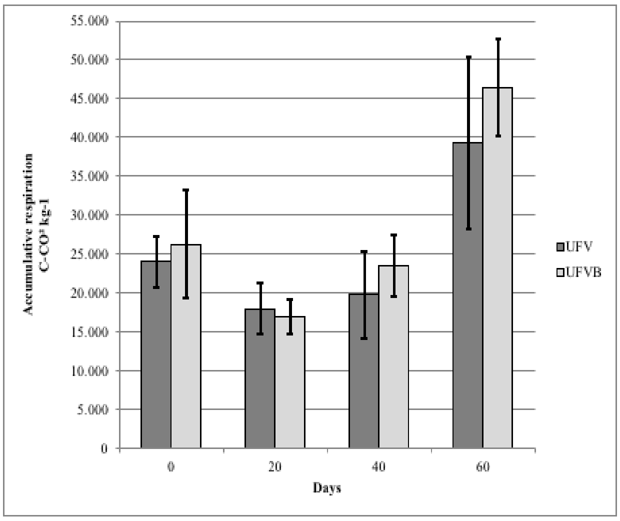

Fig. 11. Accumulative respiration.

J. *Scanning Electron Microscopy (SEM) and Micro-analysis*

Samples of two recipes were observed by means of Scanning Electron Microscopy in order to analyze their morphology: Fig. 12 and Fig. 13 display the surface of the samples at T20, showing an heterogeneous texture due to the presence of different organic and vegetal residues, both with fibrous and spherical shape.

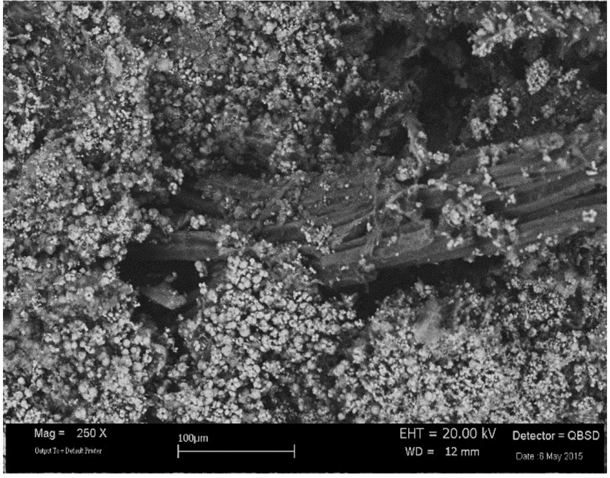

Fig. 12. SEM image of UFV recipe at T20.

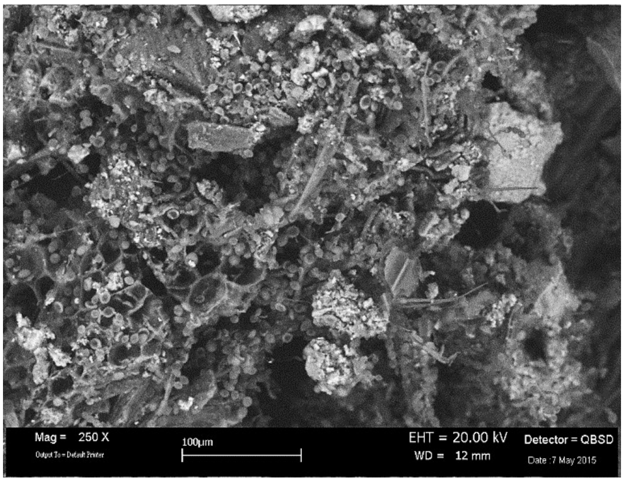

Fig. 13. SEM image of UFVB recipe at T20.

Results of the microanalyses carried out on both recipes samples at different ageing times are summarized in Fig. 14, where Carbon and Oxygen were not considered and all other elements were carried to weight percentage in order to obtain an easier comparison between them.

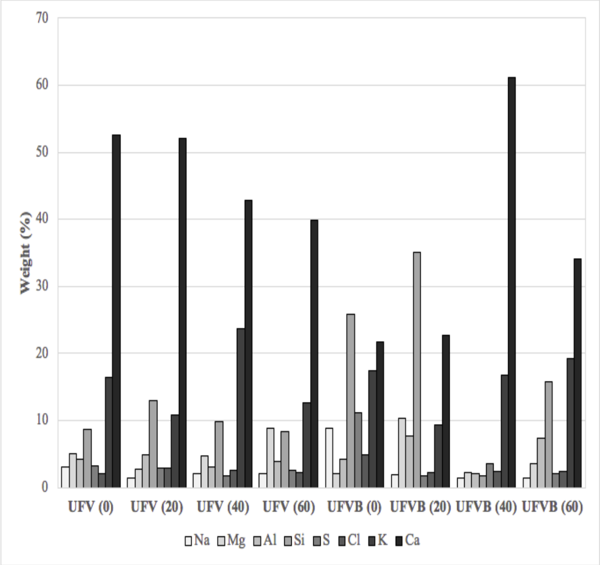

Fig. 14. Elemental microanalysis.

Major concentration of Calcium (20-60%), Silicon (10-30%) and Potassium (10-20%) were detected, while other elements like Sodium, Magnesium, Aluminium, Sulphur, Chlorine, Iron and Zinc showed traces amounts. In particular, a slight decrease of Sodium and Chlorine concentration was noticed in UFVB throughout the treatment period, while UFV showed the increase of Phosphorus concentration, according to the physical and chemical analyses.

K. *X-Ray Diffraction (XRD)*

Usually, the XRD pattern of organic based product fertilizers is characterized by an amorphous contribution and some crystalline phases. In Fig. 15 the XRD patterns for recipe UFV at time of 0, 20, 40 and 60 days of treatment have been reported: two main phases, Calcite ($CaCO_3$, ICCD card 005-0586) and Silica (SiO_2, ICCD card 046-1045) have been recognized.

At T40 and T60 these phases showed higher intensity, probably due to the reduction of the amorphous phase. Another crystalline compound was also detected after 40 days of treatment, the Struvite ($NH_4MgPO_4*6H_2O$, ICCD card 015-0762), that shows higher intensity after 60 days. This is in good agreement with the microanalyses results, that report the increase of the concentration of Phosphorus.

In Fig. 16 the XRD patterns of UFVB recipe are reported: also in these samples the XRD patterns show three main phases, recognizable as Struvite, Calcite and Silica.

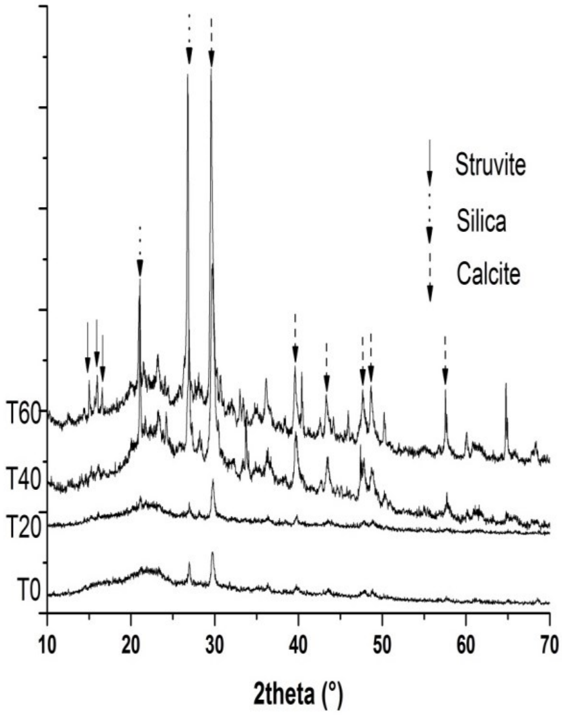

Fig. 15. XRD patterns for UFV recipe at 0, 20, 40, 60 days.

The time evolution is well represented by the presence of Struvite, which was detected with good accuracy at 40 and 60 days of treatment, while was not detected at T0. At T20 only a slight band around 14-15° (2theta) was visible, which can be interpreted as growth of Struvite.

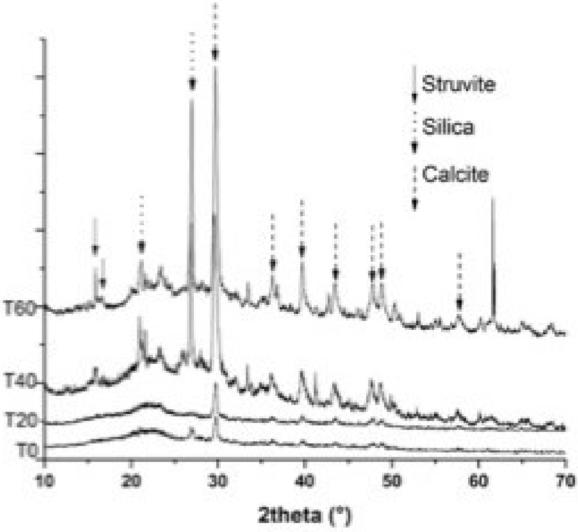

Fig. 16. XRD pattern for UFVB recipe at 0, 20, 40, 60 days.

The XRD approach was useful to obtain information about the crystalline phase of samples UFV and UFVB for each ageing time respectively, verifying the time course of the crystalline phases.

Silica and Calcite were found in both recipes: this trend agrees with the amount of Silicon and Calcium found in the microanalyses. In the UFV and UFVB compounds, the Calcite phase it is natural detected, while Silica comes from the UOW contribution to the fertilizers.

In addition, for both samples, IT-UFV and IT-UFVB, it has been noticed the reduction of the amorphous content during the ageing time.

Struvite phase, typical for aged poultry manure in which was already detected in previous works, [31] and [32], is a compound containing Ammonium and Phosphorous, which are important nutrients for the soil. In this framework, the evaluation of the presence of Struvite has been useful in order to indicate how Nitrogen and Phosphorus can crystallize.

V. CONCLUSION

Two different recipes have been compared at laboratory scale in order to obtain the best recipe to apply for pilot RESAFE fertilizer production and then for agronomic field test (UFV and UFVB, compost and dried hen manure without and with biochar respectively). They were subjected to patented enzymatic bio-treatment. Final fertilizers have an adequate organic C content in order to be considered "organic amendments". When added to the soils, they can provide all the advantages that organic matter is known for: benefits for physical properties of the soil (aggregates, bulk density, water holding capacity, porosity) [33] [34]; benefits for the chemical properties of the soil by introduction of nutrients; finally, benefits for the biological properties with the improvement of the overall system when microbial activity is added. Based on respiration results, the fertilizer processing shows the presence of different Carbon pools, with different stability degrees, useful for soil microbial life.

Both the recipes UFV and UFVB have proven to become a final organic fertilizer with a wide range of nutrients: macronutrients (N, P and K), essential element and micronutrients (B, Ca, Cu, Fe, Mg, Mn, Zn and S). This plenty of elements is very important for agriculture and can assure conditions for macronutrient efficient use.

After laboratory testing, the fertilizer obtained with 10% Biochar contribution (UFVB) was selected for application in pilot plant and further field agronomic tests. The choice was based on the high content of organic carbon and low salinity results. Salinity values were the lowest compared to the others, with final figures probably connected to mineral forms that are less water-soluble and/or linked to organic substances. It showed absolute salinity values close to the range 2 ÷ 4 mS/cm, between moderate and low salinity, as required by the project. In addition, it showed a total N content as required by the project (3%). The reduction of C_{sol} and N_{sol} after 40 days treatment, in correspondence with the detection of Struvite (Ammonium Magnesium Phosphate, $NH_4MgPO_4 \bullet 6H_2O$), could represent a mechanism to incorporate Ammonium, thus reducing both salinity and N losses via ammonia volatilization. Deeper investigations are needed, also at larger scale. Struvite, which formation was verified during poultry manure ageing [32], [31], presents low water solubility and it is a slow release Nitrogen fertilizer [35].

Finally, the use of waste and by-products in the production of organic RESAFE biofertilizer is an example of ‘closing the loop’ practices in circular economy systems, and fits the key waste management strategy of the European Community [36] of waste minimization and recycling by conversion of a by-product into marketable products [37].

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[1] The work was performed in the framework of the RESAFE, LIFE12 ENV/IT/356 project “Innovative fertilizer from urban waste, bio-char and farm residues as substitute of chemicals fertilizers”, prior to POREM, LIFE17 ENV/IT/333 “Poultry manure based bioactivator for better soil management through bioremediation”. Both projects were co-funded by EU, within the LIFE programme.

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