ANNEX 3:

POT-TRIAL PROTOCOL TO EVALUATE SHORT-TERM EFFECTS OF RECYCLING-DERIVED FERTILISERS ON THE GROWTH OF *LACTUCA SATIVA* L.

Authors: Amrita Saju, Ivona Sigurnjak, Erik Meers

Co-funder(s):

Partners:

Table of contents

1. Introduction

Experiments were conducted in controlled conditions by pot cultivation of lettuce (*Lactuca sativa* L.) to assess the RDFs and their blends as potential N fertilisers. The performance of these fertilisers were evaluated by comparing their performance with the synthetic N fertiliser calcium ammonium nitrate (CAN) with respect to crop growth, i.e. crop yield and nutrient uptake. The pot trial was performed with the aim to assess the suitability of RDFs as a substitute to synthetic N fertilisers.

2. Materials and Methods

2.1. Fertilisers

- Synthetic fertilisers (CAN for N, triple super phosphate (TSP) for P, potassium sulphate for K)
- Ammonium nitrate
- Ammonia water
- Concentrate after evaporation
- Blend 1 (ammonium nitrate + concentrate after evaporation)
- Blend 2 (ammonia water + concentrate after evaporation)

All the fertilisers were characterised for their physico-chemical parameters like dry matter (DM) and organic matter (OM) content, pH, electrical conductivity (EC), and total elemental analysis of all macro- and micronutrients (C, N, P, K, S, Ca, Mg, Na) and heavy and trace metals (Al, Cd, Pb, Ni, Cr, Cu, Co, Fe, Mn, Zn), and for their mineral N content.

2.2. Test crop

The test crop used in this trial was lettuce (*Lactuca sativa L. cv. Cosmopolia*). Seedlings of lettuce were procured from Inagro. The seedlings were watered at the rate of 1 L water minute^{-1} for a period of 5 minutes, until the time it was potted into soil.

2.3. Soil

The soil used for the pot trial was collected from the surface layer (0 - 30 cm) of an arable field in Wingene, Belgium. It is the field where the Flemish field trials for ReNu2Farm are conducted by Inagro in collaboration with UGhent. The field's soil profile is characterised as a Z.c.h. soil type (soil with sandy texture and a moderately poor drainage class with signs of rust deeper than 60 cm and a postpodzol B-horizon).

2.3.1. Soil characterisation

The soil was air-dried in the greenhouse, sieved using 2mm sieves, and mixed thoroughly for homogeneity, before performing all analyses. The air-dried soil was stored in the greenhouse in plastic bags until start of the experiment. A sub-sample of the soil was taken and the soil organic carbon (OC), pH-KCl, EC, total elemental analysis and mineral-N was performed. pH-KCl determined the soil acidity and it was measured using the Orion Star A211, Thermo Fisher Scientific, USA, by adding 25 mL of 1M KCl to 10 g of air-dried soil and letting it equilibrate for 10 minutes. EC was performed by preparing extracts of the soil in distilled water (19 g in 50 mL water) by shaking on a rotary shaker for 60 minutes. This extract was tested using an EC meter (WTW Tetra Con 96, Xylem Analytics, Germany). The soil was weighed in aluminum trays and dried in an oven at 105°C until the stabilisation of weight, after which they

were weighed again to determine the DM content from which the moisture content of the soil is determined.

Total N and total C were determined using a CN analyser (Primacs100, Skalar, the Netherlands). All the other macro- and micro-nutrients, and heavy and trace metals were analysed by preparing extractions of the soil in the aqua regia solution. 1 g of 5 sub-samples of soil were weighed in an Erlenmeyer flask. 2.5 mLof distilled water was added to moisten the samples, followed by the addition of 2.5 mL of 65% HNO_3 and 7.5 mL 37% of HCl. The flasks were covered with a watch-glass and left overnight. After12 hours, they were placed on a hot plate at 150 °C for 2.5 hours at 50 W power. After switching off the hot-plate, the flasks were kept aside to cool down. The contents of the flasks were filtered using a Whatman filter paper of pore size 125mm into a 50mL volumetric flask pre-rinsed with 1% HNO_3. These extracts were analysed on the ICP-OES (Varian Vista MPX, USA) to determine the total elemental content including macro- and micronutrients, and heavy and trace metals.

2.3.2. Field Capacity calculation

Initially, the weight of the empty pot (with geotextile pasted at the bottom) was measured. 1 kg of air-dried soil was added into this pot and the pot was weighed again. Distilled water was added into this pot and the water was allowed to leach out by gravity. It was then placed on a pot saucer and covered with a plastic sheet to avoid loss of water by evaporation. After 24 hours, the pot was weighed again, and the excess of weight from the previous day was used to calculate 100 % of the field capacity of soil. This method was followed for a triplicate set of pots and an average of the three was considered.

For lettuce cultivation, the soil was maintained at 60 % of the field water holding capacity.

2.4. Fertiliser preparation and addition

Since the fertilisers were of different texture and concentration of nutrients, they had to be prepared in a form that could be easily applied and homogenously mixed in the soil. Owing to their higher N concentration, ammonium nitrate and ammonia water would only demand a miniscule amount of application in each pot. Hence, higher concentrations of these RDFs were diluted in distilled water from which, required dosage was pipetted out and applied to the soil. Synthetic fertilisers like CAN, TSP and potassium sulphate were first ground into a fine powder and this powder was dissolved in distilled water. They were then applied from the dilutions as per the recommended dosage. Concentrate after evaporation was applied directly to the soil without any prior preparation of the product.

The nutrient recommendation considered for lettuce was 200 kg N ha^{-1}, 125 kg P_2O_5 ha^{-1} and 240 kg K_2O ha^{-1} (personal communication with Inagro). As per the recommendation, the fertilisers were added at a rate of 73 mg N, 45 mg P_2O_5 and 192 mg K_2O per pot. The pots only received a one-time application of all fertilisers.

2.5. Method for potting and further follow-up of pots

The pots used in this experiment were made of plastic with the dimensions height = 18 cm, top diameter = 13.5 cm, bottom diameter = 9.8 cm, and was filled with 1.6 kg of soil. All the pots were labelled according to their treatments, replicate number and fertiliser dosage applied. The pots were layered at the bottom with geotextile to avoid leaching of soil. Seven treatments were tested in two dosages ((100 % NPK and -50%

NPK) except unfertilised blank) with four replicates per treatment. Thus, a total of 54 pots were cultivated with lettuce in this experiment. The soil to be filled was wetted with 50% of field water holding capacity on the previous day of potting to enable easy mixing of fertilisers. The pots were first filled with 1 kg of the previously wetted soil and the fertilisers were added to the remaining 600 g soil in a container and mixed homogenously. As per the recommendation, the fertilisers were added at a rate of 73 mg N, 45 mg P_2O_5 and 192 mg K_2O per pot. This soil mixed with fertiliser was then added to the pot, the seedling was placed in this soil and water was added to make the moisture content up to 60 % of field capacity. After this, the pots were weighed and the values were noted on the label of each pot to determine water addition for coming days. The pots were then placed on a pot saucer and placed on a metal shelf where they were exposed to artificial light (Brite-grow bio-growth light) of 2000 lux for 12 hours per day at an average daily temperature of 20 °C.

The pots were watered every alternate day or depending on the loss of water detected by weighing the pots at regular intervals to maintain them at 60 % field water capacity. They were also randomized once every week.

2.6. Harvest of crops

The lettuce crops were harvested after 55 days. The plants were clipped from the roots using a scissor and soil particles, if any, were brushed off the plant biomass before weighing them to determine their fresh weight. It was then transferred to paper bags and kept in an incubator at 40 °C for 72 hours for drying, after which, the dry weight was measured. The dried plants samples were ground to a fine size using the mortar and pestle and then used for further analyses including macro- and micronutrient analyses and nitrate content, and heavy and trace elements to evaluate uptake of nutrients by plants. A sub-sample of soil from each pot was taken

to measure the moisture content, after which the remaining soil was air-dried, sieved and mixed thoroughly before analysing the soil for pH, EC, OM, macro- and micronutrients, and heavy metals.