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DETERMINATION OF NICOTINE IN THE SOIL MIXED WITH TOBACCO POWDER AS FERTILIZER

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ABSTRACT

The nicotine content of the tobacco powder completely degrades in one week when it is added to a non manured soil. If some organic manure is added to the soil so as to sensibly increase the microbial flora, then nicotine degrades in less than twenty-four hours. In sterilized soil nicotine content is constant over the time because of the total absence of microorganism. In the determination of the nicotine in the soil, accurate analytical results can be obtained by adding methanol to the soil sample at the moment of the sampling. Methanol degrades the enzymatic systems causing a soil sterilization and avoids, therefore, the

degradation of the alkaloid. The extracted nicotine is analyzed by means of high performance liquid chromatography in a rapid and accurate manner.

INTRODUCTION

In the course of the trituration of dried leaves of tobacco, necessary for the manufacturing of cigarettes, a powder deriving from the excessive mincing of the leaves is obtained; this powder, which contains over 1% in weight of nicotine, must be disposed from the tobacco manufacturers through expensive procedures because of its classification as a special and toxic waste[1]. The average chemical composition of the tobacco powder is reported in Table 1. From this data, it can be deduced that the concentration of organic substance, the favorable ratio in weight between the total carbon and nitrogen, the remarkably high concentration of phosphorus and potassium, let alone the low content of humidity, make this material suitable for a more qualified use in agriculture[2], provided that nicotine is eliminated. Our hypothesis is that nicotine can be eliminated, in an economical way, by mixing the tobacco powder with the soil, under opportune conditions. Hence it follows the necessity to have at one's disposal a procedure allowing the observation of the degradation of nicotine over the time. In the literature there are many procedures for the determination of nicotine and its metabolites in tissues[3], in biological fluids[4-6] and in tobacco[7]. Conversely, the procedures for the determination, in the soil, of alkaloids deriving from tobacco are scarce and old-fashioned. Preliminary experiments made us verify that nicotine, mixed with agricultural land, degrades quite rapidly. If manure is added to the soil, the speed of degradation increases. As a consequence, it is necessary to make the sample of soil under analysis stable, at least for the time intervening between its sampling and the conduction of the analysis in laboratory. The objective of this work is twofold: on one hand, to set a procedure that allows to take and keep the sample of soil in a simple and correct way; on the other hand, to analyze nicotine, and in laboratory, in a rapid and accurate manner.

Table 1 - Chemical and physics analysis of the tobacco powder

PARAMETER	VALUE
Moisture (105 °C)	7.7 %
pH (dil. 1:5)	5.69
Spec. el. cond.	16.3 mS/cm
Ash (550 °C)	35.4 % (dry)
Organic carbon (bichromate)	36.0 % (dry)
Organic substance (f=1.724)	62.1 % (dry)
Total nitrogen (Kjeldahl)	2.38 % (dry)
Carbon/Nitrogen ratio	15.1
Phosphorus (P_2O_5)	0.5 % (dry)
Calcium	3.7 % (dry)
Magnesium	0.55 % (dry)
Potassium (K_2O)	0.4 % (dry)
Nicotine	1.50 % (dry)

EXPERIMENTAL

Chemicals and materials

Acetonitrile, methanol and trifluoroacetic acid, all were HPLC grade (Fluka Chemie, Buchs, Switzerland), nicotine analytical grade (Fluka Chemie, Buchs, Switzerland). Water HPLC grade was produced by a Milli-Q Plus (Millipore Corporation, Bedford, MA, USA). The powder of tobacco was supplied by one of the tobacco manufacturers from the Campania region.

Apparatus

Liquid chromatograph mod. SCL 6A equipped with UV spectrophotometric detector mod. SPD-6A and electronic integrator mod.C-R6A (Shimadzu, Tokyo,

Japan). Reverse phase column with stationary phase phenyl "Luna" (Phenomenex, Torrance, CA, USA): l=250x4.6 mm; particle diameter: 5 μ; porosity: 90 Å.

Chromatographic condition

Flow: 1 mL/min; injected volume of sample: 100 μL. Eluent: A reservoir (water 100% e TFA 0,1%) and B reservoir (acetonitrile 90%, methanol 10% and TFA 0.1%. Gradient program: t=0 min B=5%; t=15 B=100%. UV detection wavelength: 265 nm.

Preparation of the calibration curve for nicotine

About 100 mg of nicotine were accurately weighed and quantitatively transferred into a 100 mL volumetric flask; the nicotine was completely dissolved in a little quantity of water and then water was added to the mark (solution A). Exactly 10 mL of the solution A were transferred in a 100 mL volumetric flask and water was added to the mark (solution B). In each of five 100 mL volumetric flasks 5, 10, 15, 20 and 25 mL of the solution B were transferred, respectively, and water added to the mark. 100 μL of each standard solution were injected into liquid chromatograph and the peak areas obtained were reported against the concentration of nicotine.

Procedure of analysis of nicotine in the soil

1) Taking of the soil sample for the analysis of nicotine

In a 100 mL-large mouth holder containing 50 mL of methanol, accurately weighed, about 50 g of soil to be analyzed were placed. The container was sealed hermetically and then weighed; by difference, the weight of the taken soil was derived.

2) Extraction of nicotine from the soil

Exactly 50 mL of distilled water were added to the container of the sample, then it was shaken for 5 minutes and the solution was let to settle. The solution

was centifuged and filtered at 0.2 μ; finally, 100 μL of the solution were injected into the liquid chromatograph. The concentration of nicotine was obtained from the calibration curve.

3) Determination of the water content of the soil

About 20 g of the soil were accurately weighed in a porcelain calibrated capsule; it was dried in a stove at 105 °C for an hour. The difference between the weight of the calibrated capsule plus the weight of sample of the soil and capsule weight after drying gives the humidity of the soil. The determination was repeated five times and the average value for the humidity was calculated.

4) Calculation

The content of nicotine in the analyzed soil can be calculated through the following equation:

$$\text{Nicotine content in soil (mg/Kg)} = \frac{100*C}{P-W}$$

where: C is the concentration of nicotine in the sample solution (mg/L); P is the weight of the sample of the soil (g); W is the water content of P g of the sampled soil (g).

RESULTS AND DISCUSSION

Degradation of nicotine in the soil

Nicotine is degraded thanks to the presence of microorganisms in the soil. To confirm this hypothesis and measure the speed of the process, a suitable quantity of agricultural land was divided into three lots. The first one was kept in a stove at 130°C for one night and, after cooling, the water eliminated during the

heating was added again to the soil. To only one of the two other lots of soil a quantity of 1% in weight of manure was added, according to the usual agricultural practice. Finally, in each of the three lots some tobacco powder, in the quantity of 1% in weight, was added and accurately mixed with the soil. Just after the preparation, three samples taken from each lot were immediately analyzed following the above-mentioned procedure. Then, the same samples were again analyzed every five hours. The results reported in Figure 1 show that in the sterilized soil the degradation of nicotine is negligible, at least over five days. In the non manured lot of soil the degradation is measurable over the time; just after one day 30% of nicotine has already degraded. In the third lot of manured land, the degradation of nicotine gets sharply more rapid. After one day, nicotine has degraded almost completely. For this reason, it is necessary to stop the degradation of nicotine in the soil at the moment of the taking of the sample, so as to obtain accurate results in laboratory.

Stabilization of the sample of soil at the moment of the taking

The degradation of nicotine is speeded up by the microbial flora that usually exists in the soil. The addition of methanol to the soil, at the moment of the taking of the sample, causes a sterilization of the soil and avoids the degradation of nicotine. To investigate the time required for the stabilization, a quantity of 1% in weight of manure and tobacco powder was added to a suitable quantity of agricultural soil. A sample was immediately analyzed, while nine other portions of soil were treated with methanol and then analyzed every five hours. The obtained results indicate that in the presence of methanol the samples can be kept at least for two days without changing the concentration of nicotine. On the other hand, during the storage, the extraction of nicotine from the soil already takes place through the action of methanol. In Figure 2 a typical chromatogram relating to a methanol extract of a soil treated with 1% of manure and tobacco powder is reported; the usual compounds of the soil and manure do not interfere with the analysis of the alkaloid.

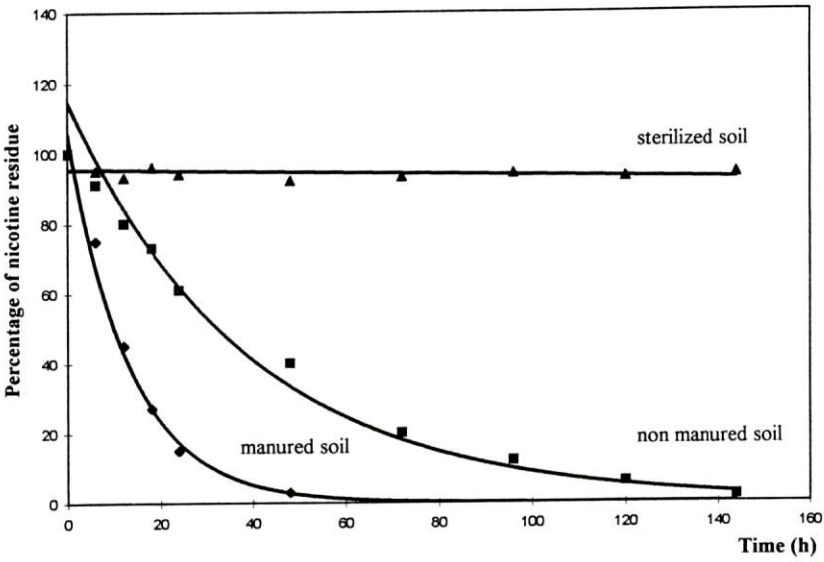

Fig. 1: Degradation of nicotine over the time in a sterilized soil, in a non manured soil and in a manured soil.

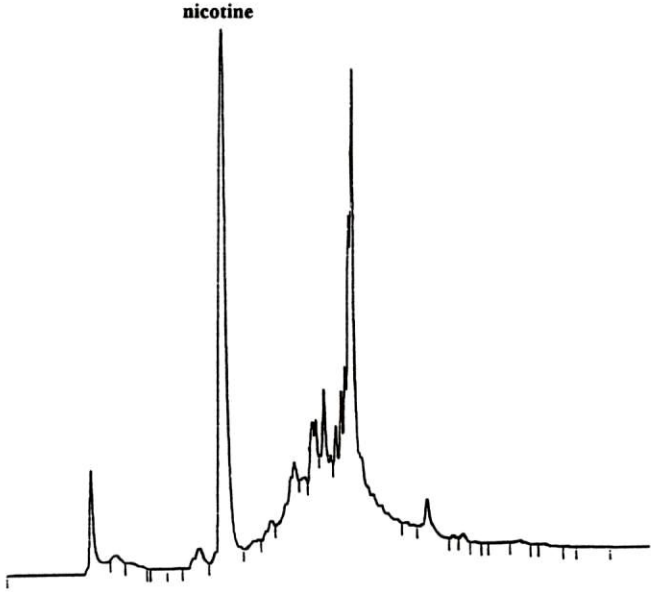

Fig. 2: Nicotine extracted with methanol from a manured soil and analyzed according to the proposed HPLC procedure.

Table 2 - Reproducibility of the analysis of nicotine according to the HPLC procedure proposed. Results of analysis of nicotine of ten samples of soil manured and added with 0.1% and 1% of tobacco powder respectively. Average values and maximum deviation from average are reported.

Sample of soil	0.1% Tobacco powder added Nicotine (mg/Kg)	1% Tobacco powder added Nicotine (mg/Kg)
1	14.2	152
2	13.9	160
3	14.4	155
4	13.5	154
5	13.8	155
6	14.0	158
7	13.9	157
8	14.1	155
9	14.1	154
10	13.7	156
Average	14.0	156
Maximum percentage deviation	2.9 %	2.6 %

Analysis of nicotine by means of HPLC

The most delicate phase of the procedure is represented by the extraction of nicotine from the soil through a solution of water and methanol. For this reason, an opportune quantity of manured soil was divided into two lots; 0.1% and 1% of tobacco powder were respectively added to the first and second lot; ten samples of the soil were taken and then they were analyzed according to the HPLC above-mentioned procedure. The results reported in Table 2 indicate that

Table 3 - Recovery of the nicotine standard added to the soil. Results of analysis of ten samples of soil manured and added with 10 and 100 mg of nicotine, respectively. Average values and maximum deviation from average are reported.

Sample of soil	0.1% nicotine added Nicotine (mg/Kg)	1% nicotine added Nicotine (mg/Kg)
1	9.9	98
2	9.8	99
3	9.6	98
4	9.7	98
5	9.3	97
6	9.8	98
7	9.7	97
8	9.4	101
9	9.6	95
10	9.8	98
Average	9.7	98
Maximum Percentage deviation	4.1 %	3.1 %

the quantities of extracted nicotine is reproducible at least in the range of experimental deviations. For the samples with 0.1% and 1% of tobacco powder the maximum deviation from the average is 2.9 % and 2.6 % respectively. To measure the recovery of nicotine, an opportune quantity of manured soil was divided into two lots; then, a quantity of 10 mg and 100 mg per 100 g of soil was added to the two lots, respectively. The soil was intimately mixed with nicotine

in order to homogenize the system. From each lot, ten samples were taken and analyzed. The obtained results, reported in Table 3, show that the recovery of nicotine from the two series of samples, after the treatment with 10 mg and 100 mg of nicotine per 100 g of soil, was quite complete.

The absorbance response at 265 nm of the nicotine in the experimental range of concentrations (0-25 mg/L) was linear with a correlation coefficient value of 0.998.

CONCLUSIONS

Nicotine in soil, particularly in the manured ones, degrades rapidly. Accurate results can be obtained by adding methanol to the soil at the moment of the taking of the sample. For concentrations of nicotine between 10 and 100 mg/g of soil, the recovery is satisfactory. The response of the nicotine for the proposed HPLC procedure is linear in the range of concentrations between 5 and 25 mg/L.

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