

## **Influence of the Application of Sewage Sludge and Presence of Pesticides on the Development of the Microbial Population of the Soil and on the Transformation of Organic Carbon and Nutrient Elements**

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**Abstract:** The laboratory trial consisted in incubating samples of soil and soil treated with sewage sludge, with the application of organophosphate pesticides with different active ingredients under controlled conditions of temperature and moisture. On the basis of a previous study of the influence of the application of sludge on the degradation of pesticides in the soil, a kinetic study is included of the degradation process and we concentrate on its effects on the development of the microbial population and the mineralization of organic carbon, together with the transformation of the main nutritive elements for plants: nitrogen and phosphorus. Three different active ingredients were used: fenitrothion, diazinon and dimethoate, all of them organophosphates with different chemical structures. From the results, it is to be observed that for all the conditions studied, degradation followed first-order kinetics. The presence of pesticides in the soil produces an increase in micro-organism populations in comparison with the control sample in the different matrices assayed, favouring the mineralization of organic carbon. As for available nitrogen, the predominant form, either ammonia or nitrates, depends on the active ingredient applied. On the other hand, the use of pesticides favours the process of mineralization/solubilization of phosphorus.

**Key words:** Sludge, organophosphate pesticides, soil, microbial population, plant nutrients

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### **INTRODUCTION**

Pesticides are chemical substances applied to the soil intentionally with the aim of killing insects, fungi or weeds. Nevertheless, once the pesticide has done its job, its continued presence in the soil is considered harmful, like that of other chemical compounds entering it via wastewater, solid residues or precipitation. Their mobility in the soil entails movement from the upper layers to the deepest ones as well as transfer to the atmosphere and draining away in groundwater and aquifers<sup>[1]</sup>.

The soil is an ecosystem with a high degradation potential, organic compounds being mainly broken down biologically, by micro-organisms, thus avoiding their accumulation in the soil and consequent environmental pollution<sup>[2]</sup>. Given the uneven distribution of micro-organisms in the soil profile, with a greater density nearer the surface, the possibility of a chemical compound being broken down decreases with depth<sup>[3]</sup>. Furthermore, the decomposition rate of chemical compounds in the soil is a function of their bioavailability to micro-organisms or enzymatic systems able to degrade them and of their amounts and activity<sup>[4,5]</sup>.

Given the biological origin of wastewater treatment plant sludges, they are very rich in biologically active

materials, which, when applied to the soil, can increase its microbiological activity and therefore, its capacity to break down residual organic pollutants. Sludge is a source of micro-organisms (bacteria, actinomycetes and fungi) capable of aerobically breaking down hydrocarbon pollutants into such harmless compounds as CO<sub>2</sub> and H<sub>2</sub>O<sup>[6]</sup>. They can also transform compounds into other, less toxic ones and/or retain them in the organic matrix and thus reduce their bioavailability. The different chemical structure of the active ingredient determines its biodegradability and bioavailability and therefore, its continued presence in the soil. In a previous study it was observed how pesticides dimethoate and fenitrothion were easily degraded thanks to the sludge addition which favoured the process. Diazinon, however, has a more complex chemical structure, belonging to the heterocyclic organophosphate group and stays in the soil longer as a result of a reducing effect of its availability<sup>[7]</sup>. The methodologies developed for analysing the active materials fenitrothion and diazinon and dimethoate are set out in previous studies<sup>[8,9]</sup>.

Pesticide residues and the products of their degradation are assimilated by the micro-organisms in the soil and by those added with the application of sludge, whereby the microbial population is increased, along with its activity, which affects the transformation

of the elements nutritive to plants<sup>[10]</sup>. Some pesticides are of no use to soil micro-organisms but can be broken down by co-metabolism<sup>[11]</sup>. It should also be borne in mind that some pesticides are harmful to soil micro-organisms.

The trial consisted in the application to the soil of dimethoate, fenitrothion and diazinon, organophosphate pesticides respectively of linear, phenolic and heterocyclic structure, with the aim of ascertaining their effects on the development of the microbial population and the mineralization of carbon, nitrogen and phosphorus. A kinetic study is also offered of the process of degradation of the different active materials in matrices of soil and soil treated with sludge in a previous study.

## MATERIALS AND METHODS

Soil was gathered in the province of León, Spain. It was taken from the top 20 cm, allowed to dry in air, broken up and sieved through a 2 mm mesh. Its physical and chemical characteristics are shown in Table 1.

Table 1: Physical and chemical characteristics of the soil

Moisture (%)	6.43
pH	5.26
Conductivity (mS cm <sup>-1</sup> )	0.03
Organic matter (%)	0.7
Organic carbon (%)	0.4
Total Nitrogen (%)	0.07
C/N ratio	6.0
Assimilable phosphorus (mg kg <sup>-1</sup> )	8.7
Calcium (mg kg <sup>-1</sup> )	226.4
Magnesium (mg kg <sup>-1</sup> )	20.6
Potassium (mg kg <sup>-1</sup> )	86.0
Sodium (mg kg <sup>-1</sup> )	2.3
C.I.C. (meq [100g] <sup>-1</sup> )	2.1
Iron (mg kg <sup>-1</sup> )	37.4
Manganese (mg kg <sup>-1</sup> )	39.7
Copper (mg kg <sup>-1</sup> )	0.6
Zinc (mg kg <sup>-1</sup> )	0.4
Boron (mg kg <sup>-1</sup> )	0.1
Sand (%)	50
Texture Silt (%)	30
Clay (%)	(sandy loam) 20

Two types of sludge were used: one, designated SS<sub>u</sub>, from an urban wastewater treatment plant with a capacity of 150,000 population equivalent, where a process of anaerobic stabilization is used followed by spinning off the water; and another, designated SS<sub>f</sub>, from a food industry treatment plant where the wastes are subjected to aerobic stabilization. The physical and chemical characteristics of the sludges are shown in Table 2.

The active materials, fenitrothion, diazinon and dimethoate, with respective purities of 96.3, 98.5 and 99.8%, were supplied by Riedel de Haën of Hannover, Germany. Figure 1 shows their chemical structure.

Table 2: Physical and chemical characteristics of the sludges

	SS <sub>f</sub>	SS <sub>u</sub>
Dry matter (%)	2.52	16.88
PH	6.05	5.66
Conductivity (mS cm <sup>-1</sup> )	9.13	3.99
Organic matter (%)	74.1	53.1
Total nitrogen (%)	6.4	6.6
C/N ratio	6.6	4.6
Total phosphorus (mg kg <sup>-1</sup> )	16700	23100
Calcium (mg kg <sup>-1</sup> )	6880	28900
Magnesium (mg kg <sup>-1</sup> )	2790	3980
Potassium (mg kg <sup>-1</sup> )	5710	3240
Sodium (mg kg <sup>-1</sup> )	15700	562
Iron (mg kg <sup>-1</sup> )	969	15100
Manganese (mg kg <sup>-1</sup> )	28	174
Zinc (mg kg <sup>-1</sup> )	138	1160
Copper (mg kg <sup>-1</sup> )	20	206
Boron (mg kg <sup>-1</sup> )	13	23
Mercury (mg kg <sup>-1</sup> )	<0.3	2.1
Lead (mg kg <sup>-1</sup> )	10.4	106
Nickel (mg kg <sup>-1</sup> )	9.4	64
Cadmium (mg kg <sup>-1</sup> )	0.3	1.7
Chromium (mg kg <sup>-1</sup> )	22	136

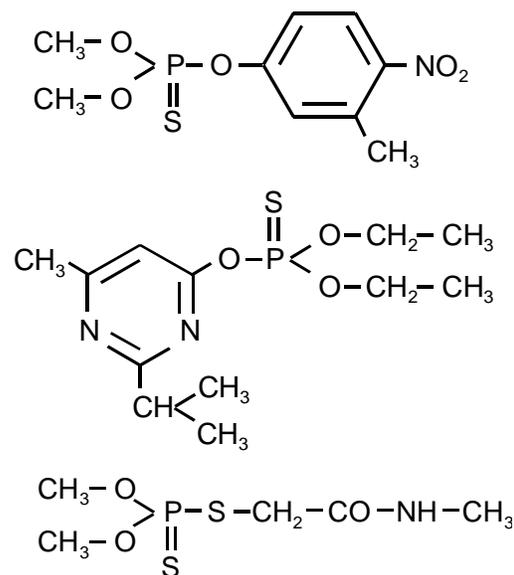


Fig. 1: Structures of fenitrothion, diazinon and dimethoate

**Preparation of samples:** Representative soil samples of 75 g were taken. Sludge was applied to each sample in turn in order to maintain the representative nature of the samples and was mixed in to achieve as homogeneous a distribution as possible. Sufficient water was added to bring the moisture content of the samples to 10%. The active material was added in a proportion of 10 µg per gram of soil, in the form of standard solutions. Incubation took place in a Sanyo Gallenkamp SGC 097-CFX-F fitotron. Temperature was fixed at 25°C and environmental humidity at 80-90%, in continuous darkness. Incubation periods were of 45 and 90 days.

**Analyses carried out in each incubation period**

**Microbial population:** The number of colony-forming units was determined by means of the plate count technique. Sowing was performed on Petri dishes with yeast extract agar as a culture medium. Incubation was effected in an oven at 37°C for 24 h, after which colonies were counted. Blank, or control, samples were also incubated, that is matrices without active materials.

**Chemical analyses:** Organic matter was determined by means of the Walkley-Black method described in the *Métodos Oficiales de Análisis* (Official Methods of Analysis) of the Spanish Ministry of Agriculture, Fisheries and Food<sup>[12]</sup>, on the basis of oxidation of organic matter. Organic carbon was determined on the basis of a ratio of organic matter content to organic carbon of 1.7241.

Ammoniacal nitrogen, extracted from the soil with a solution of potassium chloride, was measured with a METROHM 692 pH ionometer fitted with an ammonium 133/1e selective ion electrode and a temperature probe.

Nitrates were extracted from the soil with a saturated solution of calcium sulphate and determined by means of the reading of the second derivative in a BECKMAN DU 640 UV-vis spectrophotometer<sup>[13]</sup>.

Assimilable phosphorus was determined by the Olsen-Watanabe method. It was extracted with sodium bicarbonate and absorbency of the extract measured in a BECKMAN DU 640 spectrophotometer at a wavelength of 882 nm.

**RESULTS AND DISCUSSION**

**Degradation kinetics:** As the persistence of a pesticide is defined in terms of its half-life, a fit was made of the concentration and time data in order to determine the time over which concentration had decreased to 50% of its original concentration, i.e., its half-life.

In all conditions studied, it may be said that degradation followed first-order kinetics, where  $C = C_0 e^{-kt}$ ,  $C$  being the concentration of pesticide in ppm at  $t$ , time in days,  $C_0$  the initial concentration in ppm and  $k$  (days<sup>-1</sup>) the degradation constant. This agrees with other authors' results<sup>[14]</sup>. Although the degradation of some pesticides is mainly microbial, their disappearance normally follows first-order kinetics because the amount of pesticide in the soil is very small in comparison to other components. Table 3 shows the values for the degradation constant and the half-life for each active ingredient in the different matrices, along with the coefficient of linear regression ( $R^2$ ) of the previous equation transformed logarithmically.

The half-life times ( $t_{1/2}$ ), indicative of a pesticide's persistence in the soil, confirm the effect of the addition of sludge depending on the active ingredient used to treat the soil<sup>[7]</sup>. The breakdown of dimethoate and fenitrothion was aided by the application of biosolids,

Table 3: Values for the degradation constant, half-life and coefficient of regression

Fenitrothion			
	K	$t_{1/2}$	$R^2$
S	0.0482	14.4	0.9664
S+SS <sub>f</sub>	0.0568	12.2	0.9452
S+SS <sub>u</sub>	0.053	13.1	0.9361
Diazinon			
	K	$t_{1/2}$	$R^2$
S	0.0254	27.3	0.9944
S+SS <sub>f</sub>	0.0232	29.9	0.9949
S+SS <sub>u</sub>	0.0152	45.6	0.9829
Dimethoate			
	k	$t_{1/2}$	$R^2$
S	0.0941	7.4	0.9564
S+SS <sub>f</sub>	0.1478	4.7	0.8749
S+SS <sub>u</sub>	0.1431	4.9	0.9065

the kinetic constant of the process obtained in the fit increased and therefore the half-life time was reduced. For diazinon, the effect was just the opposite, sludge favouring its persistence in the soil by not aiding degradation. The degradation was mainly chemical and followed first-order kinetics.

**Development of the microbial population:** The degradation of pesticide residues in the soil and the assimilation of the degradation products brought changes to the microbial population and to the mineralization of the organic carbon and the transformation of the main nutritive elements for plants: nitrogen and phosphorus<sup>[10]</sup>.

Figure 2 shows the evolution of the total aerobes expressed as cfus per g of dry matter in samples of soil and soil treated with sludge (SS<sub>f</sub> or SS<sub>u</sub>), without pesticide and with each of the active ingredients studied; fenitrothion (ai1), diazinon (ai2) and dimethoate (ai3).

In the soil matrix the development of the micro-organism population tended to grow over time, the rate of growth depending on the active material in the sample. Diazinon did not favour the increase of total aerobes, but did inhibit growth, while the other two produced considerable growth in comparison with the control sample.

When the matrix includes sludge, there is also an increase in the population over the incubation period, greater numbers of cfus being reached than in soil without sludge, owing to the additional micro-organisms present in the sludge. The effect of the active materials is maintained, the microbial population increasing hardly at all with diazinon and increasing considerably with the other two.

In all cases, the growth of the microbial population is greater when the residue of active material in the sample is smaller and there is a greater kinetic constant of degradation ( $k$ ) and the persistence of the active material is reduced.

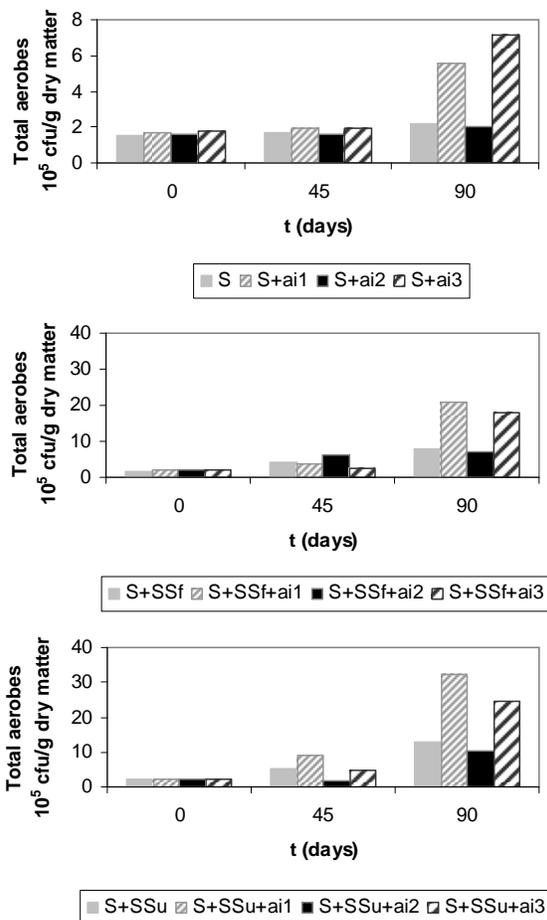


Fig. 2: Evolution of the total aerobic population in controls and samples with active materials, in the different matrices

Generally the increase in population is greatest after 45 days of incubation, when the percentage of residue in the samples is below 50% for fenitrothion and diazinon and dimethoate is no longer detectable. This indicates a greater use of the pesticide residues and the degradation products<sup>[15]</sup> by the micro-organisms to obtain energy and nutritive elements for their cell metabolism<sup>[16]</sup>.

Parallel tests were run to check the evolution of micro-organisms indicative of faecal pollution (faecal coliforms and *E. coli*). In no case was the growth of the aerobic population matched by an increase in these indicator micro-organisms.

### Transformation of organic carbon and nutritive elements

**Organic carbon:** The amount of organic carbon fell gradually throughout the test as a result of the mineralization process undergone by organic matter (Fig. 3). The incorporation of the different active materials favoured mineralization in the soil matrix, though it did not vary significantly depending on the specific pesticide used.

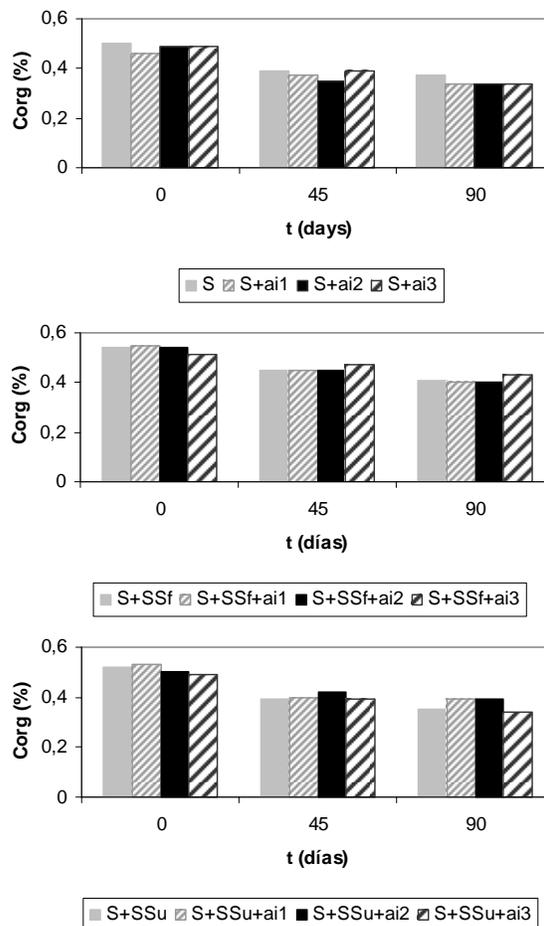


Fig. 3: Evolution of the organic carbon content of controls and samples with each active ingredient, in the different matrices

The process was also aided by the application of sludge. In the samples treated with SS<sub>f</sub> sludge, the final percentage of organic carbon was slightly lower in the samples with fenitrothion (ai1) and diazinon (ai2). On the other hand, dimethoate (ai3) in the samples with SS<sub>u</sub> sludge aided the mineralization of organic carbon more than the other active materials.

The increase or decrease in mineralization of organic matter could be due to a rise or fall in the population of the heterotrophic micro-organisms responsible for the process, or in their activity<sup>[17]</sup>, stimulated or otherwise by pesticides. These micro-organisms use organic matter as a source of carbon and energy for their cell constituents<sup>[18]</sup>, thus altering the duration of the organic carbon.

**Available nitrogen:** The evolution of nitrogen was analysed in the inorganic or mineral form, ammoniacal nitrogen and nitrates, which are the source of nitrogen for plants (Fig. 4 and 5).

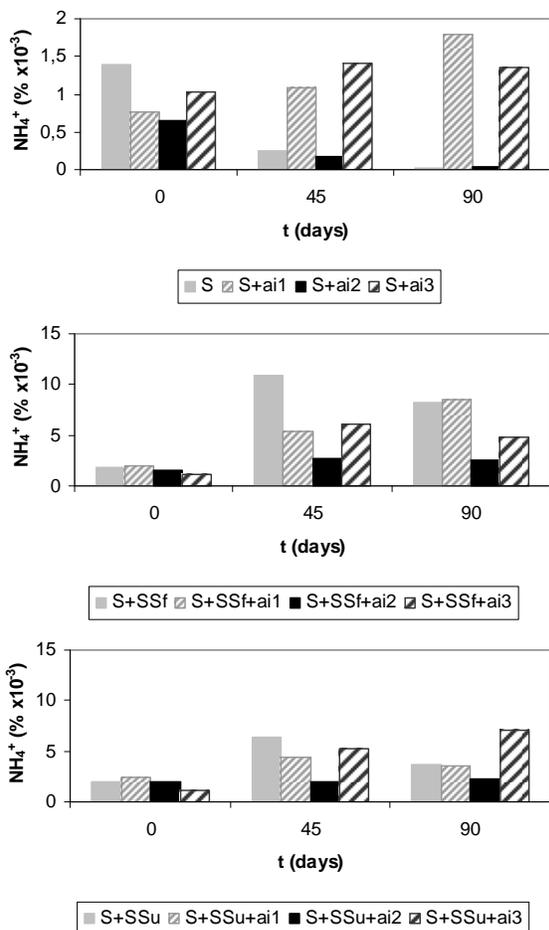


Fig. 4: Evolution of the ammoniacal nitrogen content of controls and samples with each active matter, in the different matrices

**Ammoniacal nitrogen:** Nitrogen in this form followed a general tendency dependent on the active material used. The highest percentages were found in the samples treated with fenitrothion (ai1) and dimethoate (ai3), where they increased. In the control soil, ammonia content decreased considerably, as it did in those treated with diazinon (ai2). Naturally enough, sludge in the matrix increase the levels of ammonia, despite which the control sample of soil and sludge showed and initial increase followed by a reduction, while none of the samples with diazinon showed any considerable increase.

**Nitrates:** As was to be expected, the evolution of nitrates was complementary to that of ammonia. The highest percentages of this form of nitrogen were generally found in the controls and samples with diazinon. Sludge did not increase nitrates as the inorganic nitrogen in sludge is normally in ammonia form, especially in  $\text{SS}_u$ , which underwent anaerobic digestion. Although nitrates evolved similarly regardless of the presence of sludge, the final

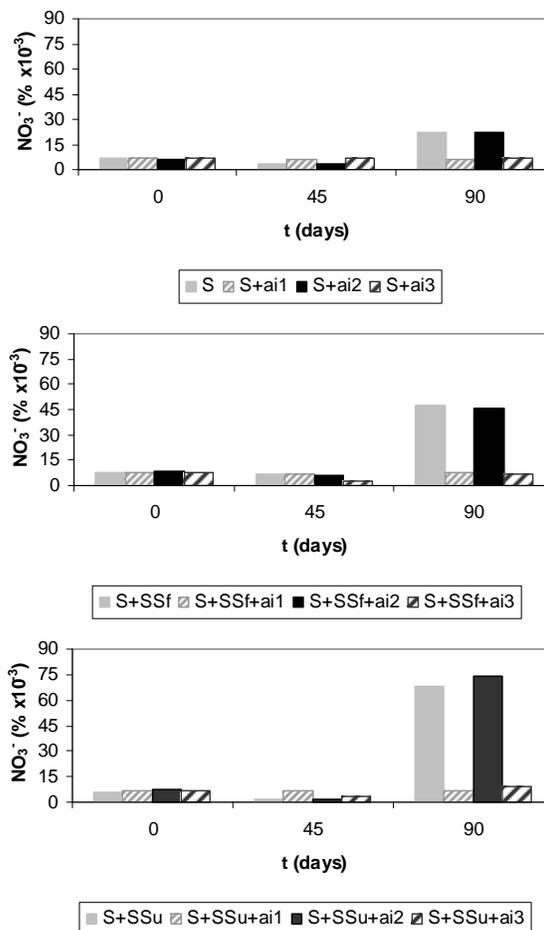


Fig. 5: Evolution of the nitrate content of controls and samples with each active material, in the different matrices

percentage increased considerably in the control sample and with diazinon, indicating a higher grade of mineralization.

The different distribution of available nitrogen in the form of ammonia and nitrates may be due to a greater or lesser development of the populations of micro-organisms with ammonifying or nitrifying activity, responsible respectively for the mineralization of organic nitrogen into ammoniacal nitrogen and the oxidation of ammonia into nitrates<sup>[19]</sup>.

Regarding pesticides, dimethoate and fenitrothion brought about a greater mineralization of nitrogen in the form of ammonia. They indirectly stimulate ammonification by destroying part of the soil's biomass, with a consequent rise in available nitrogen<sup>[10]</sup>. Diazinon did not affect mineralization in comparison with the control, aiding the formation of nitrates.

**Assimilable phosphorus:** During the incubation period, there was an increase in soluble phosphorus both in the different samples and in the controls, due mainly to the application of sludge, but the different

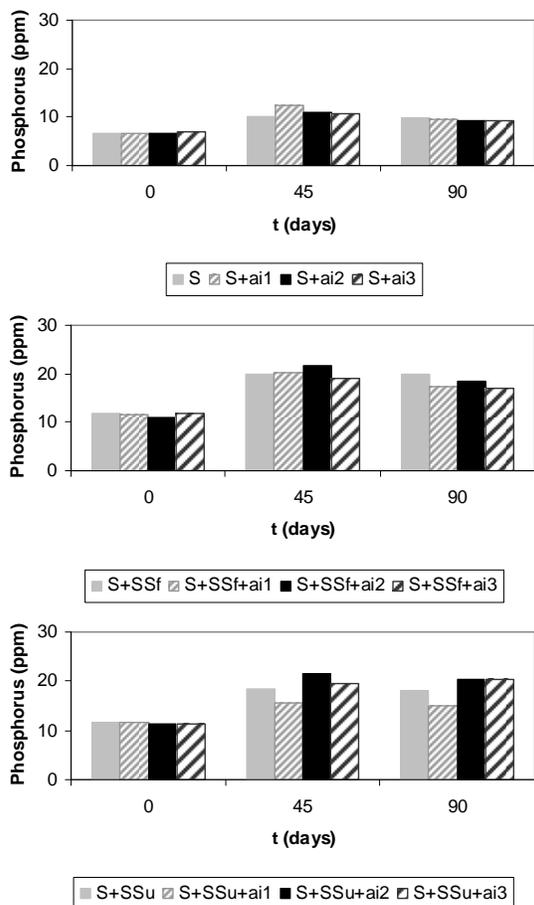


Fig. 6: Evolution of the soluble phosphorus content of controls and samples with each active material, in the different matrices

active materials produced no significant differences. In the soil and soil-plus-SS<sub>f</sub> matrices, there were no significant differences in the amounts of assimilable phosphorus for the three active materials. In the samples with SS<sub>u</sub>, which is richer in phosphorus, diazinon and to a lesser extent, dimethoate increased the amounts. Pesticides increase the amount of available phosphorus in the soil probably as a result of the increase of the micro-organisms responsible for the mineralization/solubilization of phosphates brought about by the pesticide<sup>[20]</sup>.

### CONCLUSION

The degradation of the different active ingredients follows first-order kinetics. An increase is noticed in the degradation rate and therefore a reduction occurs in the continued presence in the soil of dimethoate (ai3) and fenitrothion (ai1) with the application of sewage sludge.

In the soil matrix, all the active materials show the same tendency: an increase in total aerobes throughout

the trial. The microbial population increases with the degradation of active matter.

The application of sludge of both types studied aids the breakdown of fenitrothion and dimethoate, mainly owing to the increase in the microbial population of the sludges. Diazinon produces the opposite effect, its more complex chemical structure being hindered by the addition of sludge.

As a result of the breakdown of the pesticide residues in the soil and the assimilation of the degradation products, there is also an increase in the microbial population and in the transformation of organic carbon and the main nutritive elements for plants: nitrogen and phosphorus.

The process of mineralizing organic matter is quickened in the samples of soil and soil with sludge by the application of pesticides.

Available nitrogen is present mainly in the form of ammonia in the samples containing fenitrothion or dimethoate, but nitrates predominate in the control samples and those treated with diazinon.

The application of pesticides increases the amount of available phosphorus in the soil treated with urban sludge.

### Figure legends

- S: soil
- SS<sub>f</sub>: Sewage Sludge (from a food industry treatment plant)
- SS<sub>u</sub>: Sewage Sludge (from an urban waste water treatment plant)
- ai1: active ingredient 1, (Fenitrothion)
- ai2: active ingredient 2, (Diazinon)
- ai3: active ingredient 3, (Dimethoate)

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