Time-based evaluation of bioavailable phosphorus in a calcareous soil after the application of anaerobically digested sewage sludge

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Nowadays, phosphorus natural reserves are being depleted, while P fertilizers demand is increasing. Phosphorus is well contained in waste materials such as sewage sludge (the main byproduct obtained from wastewaters treatment). Only a small amount (1-3%) of the soil total phosphorus is bioavailable for plant nutrition. In more detail, the present study focuses on the determination of the kinetics of bioavailable phosphorus concentrations in a sandy calcareous soil after the application of sewage sludge.
A centrifuged (C) and dried (D) sewage sludges anaerobically digested were tested for fertilizing a calcareous sandy soil. 50 mL falcon tubes containing negative control (T) and soil treated with C and D were incubated from 1 to 90 days at 25 °C. Soil phosphorous fractionation was performed with the SMT method and bioavailable-P was extracted through the Olsen method. Phosphorous was spectrophotometrically quantified by the molybdovanadate method. Lastly, kinetics of bioavailable-P on soils were evaluated using four kinetic models.
Phosphorus fractions remained constant throughout the experiment. Conversely, the bioavailable-P significantly decreased from day 1 to day 90 in C and T treatment that might be due to the precipitation of P with calcium; in fact, high concentration of Ca^{2+} ions and the alkaline soil pH can induce the sequential formation of calcium phosphates, even less soluble along time. Whereas, D treatment showed a peak of bioavailable-P concentration on day 14. This trend could be due to organic carbon compounds, competitive sorption and metal bridging. The fitting of experimental data revealed that the Elovich model best described the adsorptive-precipitate process of bioavailable-P in T and C. Conversely, none of the models satisfactorily described the behavior of bioavailable-P in D samples.
This study increases the knowledge on P-related phenomena for designing and optimizing fertilizers and reducing their drawbacks such as eutrophication.

1. Introduction

Phosphorus is fundamental in plant nutrition and, in general, in many vital life processes [1]. Nowadays, phosphorus reserves are being depleted [2], while P fertilizers demand increases [3]. Furthermore, the phosphorus used in fertilizers is mainly derived from non-renewable resources (such as phosphate rocks) [4,5] with heavy environmental costs of extraction [6]. Also, it is well contained in renewable resources such as sewage sludge anaerobically digested (SSAD). To better explore this feedstock as a phosphorus source, this paper will explain how and how much of the phosphorus contained in SSADs can effectively increase phosphorus availability in soils. In other studies, it was already demonstrated that an increase in soil P could happen with SSAD addition [7]. In this new case study, the experiment is entirely focused on phosphorus changing over time. Total phosphorus (P-Tot) in soil surfaces is less abundant than nitrogen and potassium, and it can range from about 0.005 to 0.15% [8]. Moreover, the quantity of P-Tot in soils has little or no relationship with the availability for plant nutrition [9] and just a little part of this percentage (1-3%) is bioavailable [10]. In fact, plants can uptake only the inorganic forms of P as anions, namely dihydrogen phosphate ($H_2PO_4^-$) and monohydrogen phosphate (HPO_4^{2-}). The ionic form of P depends on the pH of soil solution: at pH 7, they are in equilibrium; in acid soil solution pH, $H_2PO_4^-$ is the predominant form; at alkaline pH, the most frequent form is HPO_4^{2-} [1]. P-bioavailability is related to many soil characteristics: soil texture, organic matter content, moisture content, pH, diffusion rates and relative rates of adsorption-desorption, precipitation dissolution reactions [11], soil chemical composition, amount of silicate clays, $CaCO_3$, presence of Fe or Al oxides, P addition rates and times [12] and soil temperature [13]. Soil P can be considered as accruing in three compartments:

$$\text{Solution-P} \leftrightarrow \text{Labile-P} \leftrightarrow \text{Non-labile-P} \qquad (1)$$

This subdivision is usually used to describe phosphorus pools in soils in relation to plant nutrition: Solution-P is the pool with readily available P, with soluble phosphates in soil solution; Labile-P (or Active-P) is the pool with solid inorganic and organic phosphates that are relatively easy to release in the soil solution; Non-Labile-P pool (or Fixed-P) is a source of very slow release phosphates and contains very insoluble inorganic phosphates and organic compounds resistant to mineralization. The reactions ruling this process are many: mineralization, desorption, dissolution, weathering, adsorption, precipitation, leaching, runoff and erosion. The attempt of the experiment is to determine which reactions are the most important in a controlled environment. Undoubtedly, great importance is due to the method of P quantification. Many methods are available to measure phosphorus forms in soils, but in the last years the

SMT methods (Standards Measurements and Testing) [14,15] has increased in popularity due to the repeatability, the easiness of procedures (compared to other methods) and the non-sequential extraction of P [16]. In particular, this last quality drove the choice for its application in this work. In fact, with three independent procedures, it is possible to measure P-Tot, to estimate organic phosphorus (P-Org), inorganic phosphorus (P-Inorg), apatite phosphorus (AP; the form associated with calcium) and non-apatite phosphorus (NAIP; the form associated with oxides and hydroxides of Al, Fe and Mn). There are many other methods for P fractionation [17–19] but this was the only one proposed and selected by the European Community in order to have comparable results: in fact, a lack of uniformity in methods makes difficult to compare results [20]. This technique is normally applied for P-fractionation in freshwater sediment [14,21], but it can be easily adapted and applied on soils and sewage sludges [20,22–24]. As regards bioavailable-P, many methods are well-known for quantification. Olsen method [25] is considered the benchmark of P bioavailability due to its suitability to be used with calcareous and alkaline soil [11]. Finally, this work aims to determine and understand all these different P fractions' changes over time. Although soil P chemistry has been studied more intensively than that of any other element (excluding nitrogen), quantitative predictions of phosphate concentrations in soil are still lacking [26]. Nevertheless, some kinetic studies exist, and, taking advantage of models and kinetic already determined and supported by literature, the experimental data of this work will be fitted with the equations of the models. Kinetics of P-related phenomena are certainly a key to designing fertilizers and optimizing their efficiency and, hence, reducing drawbacks (e.g., eutrophication) [27]. Although kinetic can suggest retention mechanisms, soil-P behavior's complexity makes this prospect challenging to obtain [26]. Nevertheless, it will be tried to understand and interpret the physic-chemical processes that took part in this experimental campaign.

2. Materials and methods

2.1. Sewage sludge and soil analysis

The SSADs used in this experiment came from a large-scale wastewater treatment plant (3,800,000 population equivalents) located in north-west Italy. Two types of digestates were used: a centrifuged solid digestate (C) derived from a mix between primary and secondary digestate and a dried pulverulent digestate (D), obtained by the thermal treatment at 200°C of C. Physical and chemical analysis were performed on the digestates according to Cristina et al. [28], and results are summarized in **Table 1.**

The soil used in this study was sampled in Grugliasco (TO), Italy (45°03'58.4"N, 7°35'32.9"E). It was collected within 20 and 100 cm depth, sieved at 2 mm, and sterilized at 105°C. It is a sandy, calcareous and alkaline soil, poor in macronutrients and poor in organic matter. Physical and chemical analyses were performed according to Cristina et al. [29]. Detailed results of soil analysis are shown in **Table 2**.

Table 1 Physical and chemical characteristics of the SSADs used in this work

Parameter	Unit of measure	Centrifuged (C)	Dried (D)
pH (1:10)		7.3	6.8
Electrical conductivity	mS/cm	1069	1.575
N - Tot (Kjeldahl)	% d.m.b.	6.3	5
N - Org	% d.m.b.	5.33	4.75
N - NO_3^-	% d.m.b.	<0.01	<0.01
N - NH_4^+	% d.m.b.	0.97	0.25
N - org / N - Tot	%	84	94
Dry matter	%	25.8	88.8
Humidity	%	74.2	11.2
Organic matter	% d.m.b.	63.9	64.4
TOC	% d.m.b.	37.1	37.3
C/N		5.9	7.4
Ashes	% d.m.b.	36.1	35.6
Ca	% d.m.b.	5.02	4.64
Mg	% d.m.b.	1.45	1.16

Na	% d.m.b.	0.34	0.19
K	% d.m.b.	0.39	0.18
P	% d.m.b.	6.74	6.26
Fe	% d.m.b.	3.99	3.48
Mn	mg/kg d.m.b.	268	228
Cu	mg/kg d.m.b.	406	396
Zn	mg/kg d.m.b.	849	719
B	mg/kg d.m.b.	52	41
Pb	mg/kg d.m.b.	92	79
Cr	mg/kg d.m.b.	245	217
Cd	mg/kg d.m.b.	0.8	<0.1
Ni	mg/kg d.m.b.	155	137
As	mg/kg d.m.b.	0.9	<0.1
Hg	mg/kg d.m.b.	<0.1	<0.1
Cr^{6+}	mg/kg d.m.b.	<0.1	<0.1

Table 2 Physical and chemical characteristics of the sandy soil used in this work.

Parameter	**Unit**	**Value**
Stones	-	absent
Sand (2.0 - 0.020 mm)	%	94 ± 2
Silt (0.020 - 0.002 mm)	%	3 ± 1
Clay (< 0.002 mm)	%	3 ± 1
Texture	-	sandy
pH	-	8.2 ± 0.16
Electrical conductivity	dS/m	0.131 ± 0.018
Organic matter	%	0.38 ± 0.12
Organic carbon	%	0.22 ± 0.07
N - Tot (Kjeldahl)	g/kg	0.29 ± 0.09
N - NO_2^-	mg/kg	< 0,2
N - NO_3^-	mg/kg	6.33 ± 1.53
N - NH_4^+	mg/kg	3 ± 1
N - Org	g/kg	0.29 ± 0.09
C/N		7.6 ± 0.2
P - Olsen	mg/kg	1.8 ± 1.3
K$_{\text{exchangeable}}$	mg/kg	18 ± 1
Mg$_{\text{exchangeable}}$	mg/kg	15 ± 5
Ca$_{\text{exchangeable}}$	mg/kg	675 ± 27
Na$_{\text{exchangeable}}$	mg/kg	6 ± 3
Fe$_{\text{available}}$	mg/kg	6.7 ± 1.1
Mn$_{\text{available}}$	mg/kg	6.5 ± 3.0
Cu$_{\text{available}}$	mg/kg	0.69 ± 0.29
Zn$_{\text{available}}$	mg/kg	0.47 ± 0.29
CEC	cmol/kg	3.65 ± 0.35

2.2. Methods of phosphorus analysis in soil and sewage sludge

Phosphorus in SSADs and soil was extracted using the SMT method (Standards, Measurements and Testing Programme), a standardized method developed and approved by the European Commission [14,15]. Basically, it is a modified version of the Williams protocol [30] and it allows, with three independent procedures, to extract five P fractions: total phosphorus (P-Tot), inorganic phosphorus (P-Inorg), organic phosphorus (P-Org), non-apatite inorganic phosphorus (NAIP) and apatite phosphorus (AP). For P-Tot extraction, 200 mg of dry sample were calcinated in porcelain crucible at 450°C for three hours. Once the porcelain crucible was cooled down, ashes were transferred into centrifuge tubes and 20 ml of 3.5 mol L^{-1} HCl were added. After that, centrifuge tubes were covered and stirred overnight (all overnight stirrings were performed for 16 hours). After 16 hours, samples were centrifuged at 2000 g for 15 minutes. The extract was collected and analyzed for determining P-Tot. Inorganic phosphorus was determined by adding 20 ml of 1 mol L^{-1} HCl to a centrifuge tube containing 200 mg of dried sample of SSADs or, alternatively, of treated sand. After an overnight stirring, the tubes were centrifuged at 2000 g for 15 minutes. The extract was used to determine P-Inorg and the residue was used for P-Org extraction. In fact, 12 ml of demineralized water were added two times to wash the residue, centrifuged at 2000 g for 15 minutes and the supernatant was discarded. The residue was dried at 80°C and then transferred to a porcelain crucible. After calcination of 3 hours at 450°C, ashes were put into centrifuge tubes with 20 ml of 1 mol L^{-1} HCl. Centrifuge tubes were closed and stirred overnight. After an overnight stirring of 16 hours, the tubes were centrifuged at 2000 g for 15 minutes and the extract was collected and analyzed for P-Org determination. In order to extract AP and NAIP a sequential extraction procedure was applied: firstly, 20 ml of 1 mol L^{-1} NaOH were added to 200 mg of dried sample. After stirring overnight, samples were centrifuged at 2000 g for 15 minutes. From this point, 10 ml of the extract were collected and used for NAIP extraction while the cake of centrifugation was used for AP extraction. To the 10 ml of the extract were added 4 ml of 3.5 mol L^{-1} HCl and then stirred for 20 seconds. The samples were let stand for 16 hours in a covered centrifuge tube, centrifugation at 2000 g for 15 minutes was applied. NAIP was measured on the supernatant. The cake of extraction previously obtained was used for AP extraction. For two times were added 12 ml 1 mol L^{-1} NaCl then stirred for 5 minutes, then centrifuged at 2000 g for 15 minutes and finally discarded the supernatant. After that, it was added 20 ml of 1 mol L^{-1} HCl to the solid part and the tube was covered and stirred overnight. After centrifugation at 2000 g for 15 minutes, the extract was used for apatite phosphorus measuring. The accuracy of results obtained with the protocol was tested by comparing the sum of single fractions as suggested by Xie and colleagues [24]:

$$\text{P-Tot} = \text{P-Inorg} + \text{P-Org} \text{ and } \text{P-Inorg} = \text{NAIP} + \text{AP} \quad (2)$$

Available-P (Olsen-P) was extracted using Olsen method [25], a procedure suitable for both acidic and alkaline soils, and also for soils with a high presence of $CaCO_3$. Following the method, two grams of dried sample were injected into a plastic container. Then were added 0.5 g of activated carbon and 40 mL of 0.5 mol L^{-1} of $NaHCO_3$ (with a pH of 8.5). Previously, the activated carbon was washed with the solution of sodium bicarbonate in order to eliminate residual P on the activated carbon. Afterward, the plastic container was kept in agitation for 30 minutes and the solution was filtered with filters Whatman® n° 42. 20 ml of the liquid solution were collected in a plastic tube and 10 ml of H_2SO_4 1 N were slowly added. After 24 hours from the addition of H_2SO_4, the bioavailable-P contained into the partially covered tube were determined. The method relies on the presence of carbonate ions in the solution that reduces the activity of Ca^{2+} and Al^{3+} with a consequent increase of phosphorus solubility. In calcareous soils, the addition of carbonate ions reduces the activity of Ca^{2+} which precipitates in the form of $CaCO_3$ and, in this way, the solubility of the calcium phosphate increases with the decrease of the Ca^{2+} in solution.

Phosphorus quantification, in the form of orthophosphate PO_4^{3-}, was determined spectrophotometrically at 430 nm using a Hach DR5000 spectrophotometer through the Hach molybdovanadate method (Yellow method - 8114) adapted from Standard methods for the examination of water and wastewater [31]. With this method, the PO_4^{3-}, reacts with molybdate in an acid medium to give a phosphomolybdate complex. Vanadium in the sample causes a yellow vanadomolybdophosphoric acid to form. The intensity of the yellow color is proportional to the phosphate concentration. All reagents used had high purity (>99.5%) and all glassware, plasticware and laboratory tools were cleaned with HCl 6M, rinsed 3 times with deionized water, one time with milliQ water in order to remove all impurities and finally air-dried at 60°C.

2.3. Climatic chamber

A climatic chamber was built in order to have a constant temperature. An insulating box was used to thermally insulate the system and a Peltier cell was exploited for the purpose of maintaining the chamber at a constant temperature of 25°C ± 0.2°C. In addition, a beaker full of water was left into the chamber for keeping an elevated relative humidity into it. A thermometer and a hygrometer were indeed placed inside the chamber for monitoring temperature and humidity percentage; all these data were measured and recorded into an SD-card every 2 minutes.

2.4. Experimental part

The soil was collected, sieved at 2 mm and air-dried at 105°C for 48 hours. After that, three different treatment were performed: in C case, 3 kg of sandy soil were mixed with centrifuged digestate (C); in D case, 3 kg of sandy soil were mixed with dried digestate (D); in T case 3 kg of sandy soil were used as control (T). After that, digestates treatments were thoroughly and accurately mixed. Finally, 70 g of the mixed sand were inserted into sterilized plastic tubes and milliQ water was added to each plastic tubes in order to reach field capacity according to Benton Jones [1]. The concentration of P-Tot added with treatments was estimated in about 100 ppm of P in order to be around 200 kg of P_2O_5/ha application rate. Samples were incubated in the climatic chamber for 1, 3, 7, 14, 28, 45, 60 and 90 days. At these times, three replicates of each treatment were randomly selected and sampled. The central part of the sand contained in the plastic tubes was firstly air-dried at 105°C and then P-Tot, P-Inorg, P-Org, NAIP, AP and bioavailable-P were evaluated. A completely randomized disposition was used for plastic tubes in the climatic chamber. All samples stayed at 25°C ± 0.2°C and relative humidity was between 90.0% and 99.9%. Every seven days all samples were weighted and take back to the field capacity thanks to new milliQ water addition.

2.5. Kinetic modeling

Kinetics of bioavailable-P (kinetic of $NaHCO_3$ extractable-P) on soils with T, C and D treatments were analyzed using four kinetic models: first order, second order, Elovich and power function equations according to Sparks and Sinegani [13,32] and summarized in **Table 3**. Excluding the power function equation, in all other equations were applied the boundary conditions $t = 0$ to $t = t$ and $P_t = P_0$ to $P_t = P_t$ in which t represents the time and P_t the bioavailable phosphorus.

Table 3 The kinetic equations tested in the present work

Model	Linear form	Equation
First order	$\ln P_t = \ln P_0 - k_1 \cdot t$	$P_t = \frac{P_0}{1 + k_2 \cdot P_0 \cdot t}$
Second order	$\frac{1}{P_t} = \frac{1}{P_0} + k_2 \cdot t$	$P_t = P_0 \cdot e^{-k_1 \cdot t}$
Simple Elovich	$P_t = \frac{1}{\beta} ln(\alpha\beta) + \frac{1}{\beta} ln\, t$	$P_t = \frac{1}{\beta} ln(\alpha\beta) + \frac{1}{\beta} ln\, t$
Power function	$\ln P_t = \ln a + b \cdot \ln t$	$P_t = a \cdot t^b$

Usually, first-order equation is expressed as [32] in equation (3):

$$\frac{d(P_t)}{dt} = -k_1 \cdot (P_t) \tag{3}$$

P_t represents the bioavailable phosphorus at time t expressed in PPM and k_1 (expressed in h^{-1}) represents the rate constant of first-order sorption. The integrated form of equation (3) is expressed in equation (4):

$$ln\, P_t = ln\, P_0 - k_1 \cdot t \tag{4}$$

Second-order kinetic equation is expressed as [32] in equation (5):

$$\frac{d(P_t)}{d_t} = -k_2 \cdot (P_t)^2 \tag{5}$$

P_t represents the bioavailable phosphorus at time t expressed in PPM and k_2 represents the rate constant of second-order sorption. The integrated form of equation (5) is expressed in equation (6):

$$\frac{1}{P_t} = 1/P_0 + k_2 \cdot t \tag{6}$$

Elovich kinetic equation is expressed as [32] in equation (7):

$$\frac{d(P_t)}{d_t} = \alpha e^{-\beta \cdot (P_t)} \tag{7}$$

P_t represents the bioavailable phosphorus at time t expressed in PPM; α represents the initial adsorption rate and β is the desorption constant [13]. Assuming that $\alpha \beta t >> 1$ [33] the equation becomes as expressed in equation (8):

$$P_t = \frac{1}{\beta} \cdot \ln(\alpha\beta) + (\frac{1}{\beta}) \cdot lnt \tag{8}$$

Power function equation is expressed as [32] in equation (9):

$$(P_t) = at^b \tag{9}$$

P_t is the bioavailable phosphorus at time t; a and b are equation constant [13]. The linearized form is expressed in equation (10):

$$Ln(Pt) = lna + blnt \tag{10}$$

All equations used to describe kinetics were evaluated according to their coefficient of determination (r^2) and standard error of the estimate (SE) [8,27,32,33]. Due to the no systematic attempt of literature in the interpretation of the parameters resulted from P kinetics [34], these parameters data will be not showed.

2.6. Statistical analysis

One-way ANOVA was performed separately on P-Tot and bioavailable-P results of control, centrifuged and dried treatments. After the ANOVA, Tukey's post-hoc test ($P < 0.05$) was performed. The statistical software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analyses.

3. Result and discussion

3.1. Total phosphorus

Total phosphorus was measured on the sandy soil and on SSADs before the experiment. P-Tot in sandy soil was 478 ± 12 ppm, a normal value if referred to calcareous soils [27]. Phosphorus in centrifuged SSAD resulted 2.82 ± 0.02% (d.m.b.). It means that the P_2O_5 contained was 6.45% because of the conversion factor of 2.291 ($2.82 \cdot 2.291 = 6.45$). Phosphorus in dried SSAD was 2.98 ± 0.01% (d.m.b.), which means that the P_2O_5 contained was 6.84%. In **Fig. 1** the time course of phosphorus in soil no treated (control) and treated with centrifuged and dried SSADs is shown. Firstly, it worth noting that, from day 1, there was an increment of phosphorus of around 100 ppm in C and D thanks to the addition of 200 Kg of P_2O_5/ha of digestate treatments. Apparently, PPM mean values changed in different times of sampling. The statistical analysis allowed to reject this possibility (**Fig. 2**). In fact, the Tukey test revealed that no significant changes occurred during experiment time. Total P remained constant for 90 days in control soil (**Fig. 2a),** in soil with centrifuged SSAD (**Fig. 2b**) and in soil with the dried digestate (**Fig. 2c**). This trend revealed as expected and in line with the literature (100-3000 ppm [35]). In fact, phosphorus has got a very low solubility in soil system (<0.01 mg P L^{-1} [35]). It could be lost by erosion and runoff or absorbed by plants, all phenomena that could not appear in a closed system such as the falcon tubes used in this experiment. In the closed system designed for this experiment it could be "lost" only for leaching, moving from the upper part of the tube to the lower part: anyway, statistical analysis disclaim this hypothesis (data not shown).

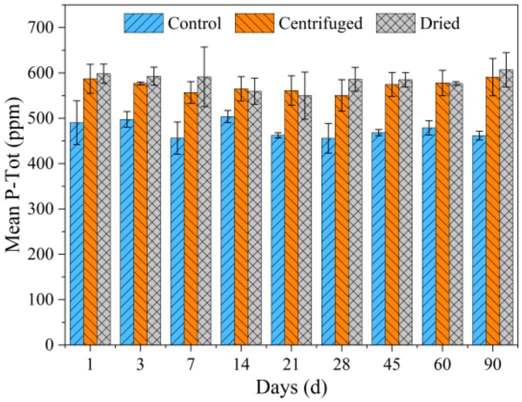

Fig. 1 Mean P-Tot (ppm) with different treatments among three months. Each error bar represents the standard deviation

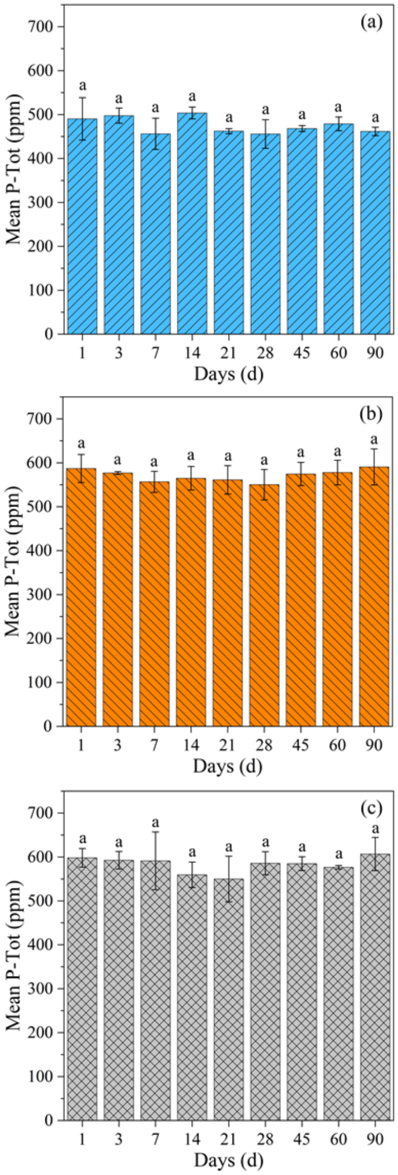

Fig. 2 Mean P-Tot (ppm) in soil of control (**a**), centrifuged (**b**) and dried (**c**) among three months. Different letters indicate differences between ppm levels that are significant at $P < 0.05$ (Tukey HSD). Each error bar represents one standard deviation

3.2. Phosphorus fractionation

The fractionation of the P contained in SSADs revealed that most of it was in the inorganic form: 85.3% (24033 ppm) in centrifuged digestate and 83.9% (25050 ppm) in dried digestate. P-Org was 9.9% (2779 ppm) in centrifuged and 7.1% (2107 ppm) in dried digestate (**Fig. 3a**). In centrifuged digestate there was a missing percentage of 4.8% while in dried SSAD was about 9.0%. Due to the sequential extraction process of the method, the SMT usually gives not residual parts of P [14]. On the other hand, it is possible to broadly compare these results to the others in literature since many other studies on SS gave similar residual parts to the ones obtained in this study. A study on different forms of P contained in sewage sludge of 46 cities and measured with the SMT method, revealed that more than 60% of P-Tot was inorganic and about 26% was organic [36]. Another work that used SMT method [24] reported that in their sewage sludges P-Inorg ranged from 75.9 and 87.6% of P-Tot and P-Org ranged from 11.5 and 22.1% of P-Tot. Garcia-Albacete and coworkers analyzed heat SS with SMT method and revealed that 87.6% of P was inorganic and 2.56% was organic [20]. This comparison revealed that results obtained on SS are in line with other literature studies, with P-Inorg >> P-Org. The different proportions of P fractions may depend on the origin and the treatment process of sludge [37]. Taking into account this last assertion, the two SSADs differ for the heat treatment. It may have changed the proportion between P-Inorg and P-Org: in fact, probably because the heat treatment both the P-Org total content and the P-Org percentages were lower in Dried SSAD [37,38].

Concerning NAIP and AP in SSADs the application of SMT method gave an overestimation of results probably due to an underestimation of P-Inorg: in fact, the sum of NAIP and AP should give P-Inorg. In this study, there was an overestimation of 2.5% in centrifuged SSAD and 5.4% in dried SSAD. Mean percentages of NAIP referred to P-Inorg were 68.6% (16497 ppm) and 79.4% (19909 ppm) in centrifuged and dried SSADs respectively. Mean percentages of apatite phosphorus related to the inorganic one were 33.9% (8137 ppm) and 25.9% (6499 ppm) in centrifuged and dried digestates respectively (**Fig. 3b**). As before, it is possible to broadly compare this work to the ones in the literature, since other studies show similar residual parts. The research on the sludges of 46 cities cited above [36] mentioned that the mean percentage (referred to P-Inorg) of NAIP and AP were about 78% and 26% respectively. Xie et al. [24] referred that in their SS NAIP ranged from 32.4% to 68.0% of P-Tot and AP ranged from 37.1% to 69.7%. The work of Garcia-Albacete and colleagues revealed that 32.0% of inorganic P was NAIP and 51.5% was AP [20]. In Nobile and colleagues work [37], a dewatered SSAD had 75.3% of AP and 4.8% of NAIP; in the same work the same SSAD received a heat treatment that changed that proportion to 63.4% of AP and 18.7% of NAIP. That different proportion of AP and NAIP could be due to different chemical and thermic treatments in WWTPs. For what it concerns chemical treatments, a large proportion of AP may be attributed to the addition of lime to improve dewatering efficiency during the sludge treatment process [24], while, on the contrary, a high presence of NAIP could be related to the iron content of the sludge [20]. Such as before, it is possible that the different percentages between centrifuged and dried SSADs in terms of NAIP and AP could be motivated by the heat treatment of the dried SSAD [37,38]: it was demonstrated that in sludges the heat treatment between 120° and 220°C could increase NAIP % and decrease AP %. Above 220°C, NAIP and AP exchange rapidly their percentages [38]. SSADs used in this work derived from the same raw material; dried SSAD derived from centrifuged digestate thermally treated at around 180°C: this treatment probably increased P-Inorg % and NAIP %.

Unfortunately, the application of SMT method to treated and non-treated soils did not give the expected results. Sum of P-inorg + P-Org did not reach P-Tot. If in SSADs there was an "acceptable" "missing part of P" (<9%), during three months of soil study the "missing P" had an average percentage of 23.7%, 26.0% and 19.9% in control, centrifuged and dried samples respectively. Although some authors revealed that is normal to have an underestimation of the values of P-Inorg + P-Org with respect to P-Tot (between 0 and 22% [20,21]), it is not correct to find any variation on these P fractions. By the way, in **Fig. 4** the time course of P-Inorg and P-Org fractions compared to P-Tot is represented. Literature supports the thesis that, after the addition of sludge to the soil, the transformation of P-Org contained in sludge into P-Inorg can be occurred [39]. Concerning NAIP and AP changes during the three months of the experiment, measures were recorded in day 1, 28, 60 and 90. Nevertheless, the sum of average values of NAIP + AP was 96.1%, 98.8% and 86.3% of P-Inorg mean values measured on control, centrifuged and dried soils respectively, and, for that reason, it is not correct to comment these data. In fact, if there was an underestimation of P-Inorg, it was also possible that NAIP or AP was underestimated. Anyway, a graphical representation of the sum of NAIP + AP compared to P-Inorg is reported in **Fig. 5**.

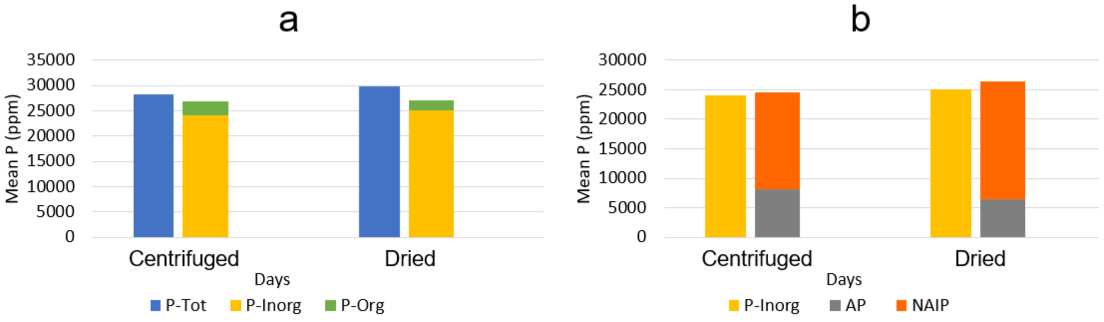

Fig. 3 P fractionation in centrifuged and dried SSAD. **a**: P-Tot fractionation in P-Inorg and P-Org; **b**: P-Inorg fractionation in AP and NAIP

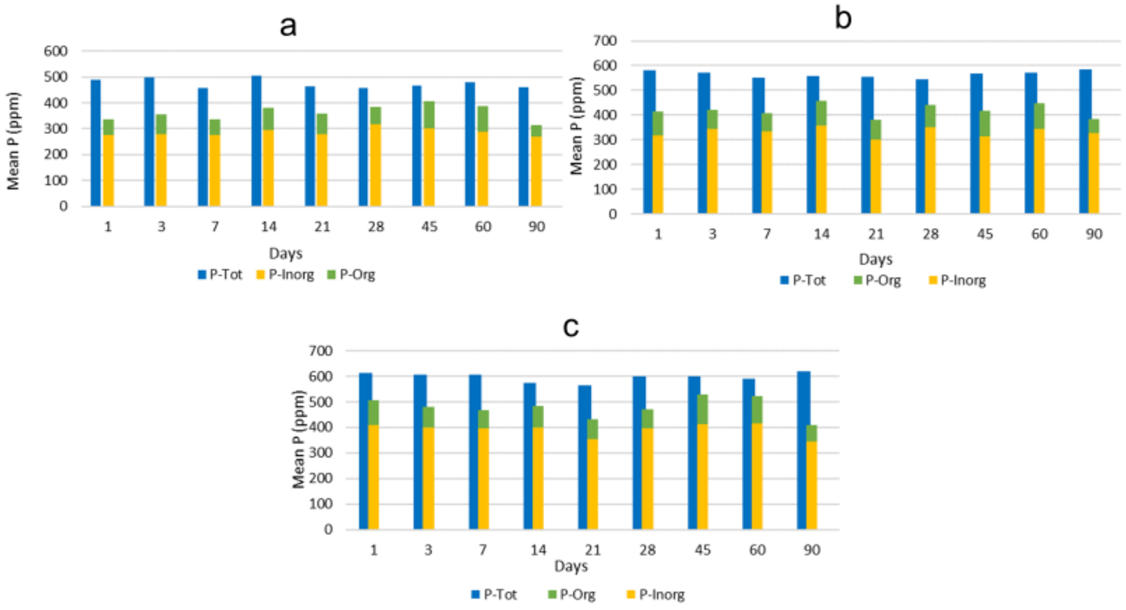

Fig. 4 Sum of mean values of inorganic and organic P fractions compared to mean values of P-Tot during 3 months of the experiment. **a**: the time course of control. **b**: the time course of C treatment. **c**: the time course of D treatment

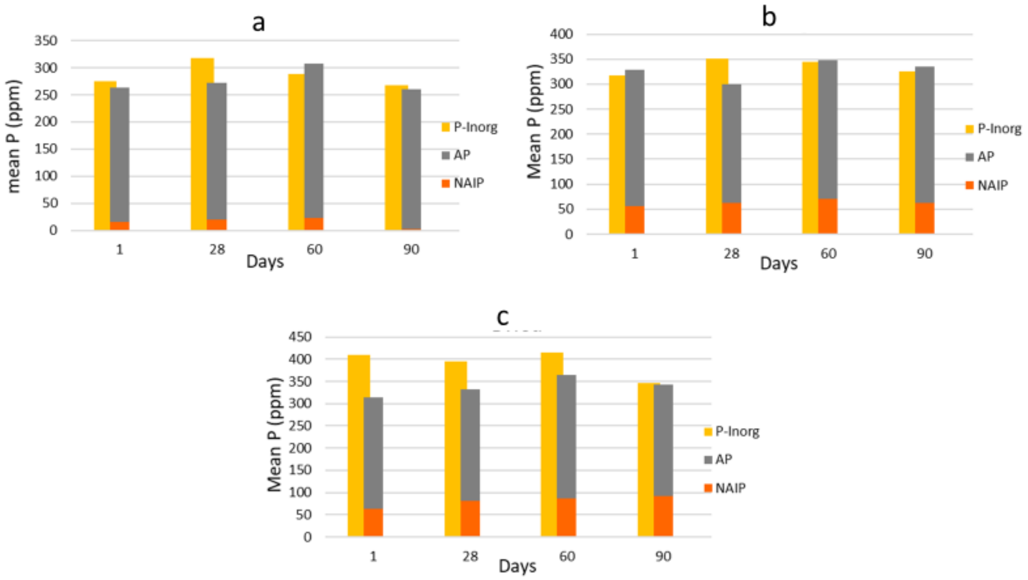

Fig. 5 Sum of mean values of NAIP and AP fractions compared to mean values of P-Inorg during 3 months of the experiment. **a**: the time course of control. **b**: the time course of C treatment. **c**: the time course of D treatment

3.3. Bioavailable-P

Bioavailable-P ($NaHCO_3$ extractable P) in soil before the experiment was 4.4 ± 1.3 ppm. This is a very low value if compared to other soils [40]. Considering that the extraction of bioavailable-P with the Olsen method is appropriate and precise only in soils, bioavailable-P was not extracted on SSADs. On the other hand, some authors assume that the bioavailable-P can be represented by the sum of NAIP and P-Org (only partially available) measured with SMT method [16,24,41]: in this way, the 68.5% and the 73.8% of P-Tot of centrifuged and dried SSADs con be considered as potentially bioavailable. Surely, due to the residual-P not determined in P fractionation, it is not correct to compare these two percentages. It is only possible to observe that, using SMT method, most of the P contained in SSADs can be considered bioavailable. Furthermore, because of the different methods of analysis, this data can't be directly compared to Olsen ones. **Fig. 6** shows the time course of average P-bioavailable during three months of the experiment. Data analysis revealed that the SSAD addition to soil increased the bioavailable-P. This result confirms the fertilizing properties of SSADs and it supports the results obtained in treated soils in the previous experiment (Chapter III). This increase is supported by other studies in the literature [34,37,42]. The day after the addition of SSADs (Day 1), the quantity of mean bioavailable-P increased from 4.4 ± 1.3 ppm of the not-treated soil before the addition of SSAD to 34.9 ± 2.9 ppm of centrifuged-treated soil and to 22.2 ± 1.5 ppm of dried-treated soil. Considering that 100 ppm of P-Tot were added with SSADs application, a great percentage of that can be considered as bioavailable: around 30% and 18% in centrifuged and dried SSADs, respectively. These values were lower than was expected to be bioavailable-P calculated as the sum of NAIP and P-Org. This difference can be due to two different reasons: firstly, they were extracted with two different methods and, secondly, they were extracted after one day from P addition and a loss of bioavailable-P could have happened meanwhile. In the following days, a decrease in bioavailable-P in control and centrifuged treatments seemed to happen. Statistical analysis confirmed these decreasing trends (**Fig. 7**). **Fig. 7a** shows the bioavailable-P gradual decrease in control soil from 4.2 ± 1.2 ppm of day 1 to 0.3 ± 0.6 ppm of day 90. **Fig. 7b** shows the bioavailable-P decrease of soil treated with centrifuged SSAD: it decreased from 34.9 ± 2.9 ppm of day 1 to 23.8 ± 1.5 ppm of day 90. Similar decreasing of bioavailable-P over time is well supported in the literature: in fact, similar decreasing trends were found using organic soil improvers (such as farmyard manure, processed city waste and poultry litter [43]) and using mineral fertilizers such as KH_2PO_4 [13,44]. The decrease in P-bioavailability could be motivated by many processes: immobilization, adsorption, precipitation, leaching, runoff and erosion. Runoff and erosion must be excluded because of the closed system of the experiment. The measure of bioavailable-P in the upper, medium and lower parts of the samples demonstrated that leaching did not appear (data not shown). Immobilization, due to the sterilization of sandy soil at 105°C, should be excluded at least in control; on the other hand, it is possible that some microorganisms survived in centrifuged and dried SSADs. Moreover, net immobilization can occur if the substances added to soil have a C/P ratio greater than 300:1, while net mineralization is likely if the ratio is below 200:1 [45]. The C:P ratio of SSADs was below 200. Hence, it was also possible that the mineralization process appeared causing an increase in P availability, but, due to the non-increase of bioavailable P, this option was not observed here. The most probable processes that could have happened are adsorption and precipitation. Different trend appeared in the bioavailable-P of dried SSAD **(Fig. 7c**). Data analysis highlighted that, from day 1 (22.2 ± 1.5 ppm) to day 90 (the lowest mean value, 19.6 ± 0 ppm), there was a peak on day 14 in which the concentration of bioavailable-P was the highest (26.6 ± 3.0 ppm). This different trend could be due to the presence of organic matter (OM) in SSADs. It is known that OM can enhance P bioavailability: after addition of OM to soil, there is an inhibition of P sorption thanks to dissolved organic carbon compounds - DOC (humic acids, fulvic acids, tannic acids, aromatic acids, low molecular weight organic acids, polysaccharides, amines and others C compounds [46]). DOC can release phosphorus by decreasing sorption sites on colloid surfaces by metal complexation, dissolution reaction (on Fe and Al oxides), competitive sorption and metal bridging [47]. In soil treated with dried SSAD, probably the DOC contained in the sludge acted slowly with one or more of these modalities. However, this explication does not clarify why the same phenomenon did not appear in soil with centrifuged SSAD. It is possible that in C treatment the effects of OM addition were rapid (in the first 24 hours), while in D treatment, these reactions were postponed due to the heat treatment. Another conceivable hypothesis to justify this peak may be a possible anoxic condition in the sample analyzed: in fact, the release of P is favored under anaerobic conditions as a consequence of Fe reduction. All these hypotheses on bioavailable-P changed over time will be better explored in the next paragraph.

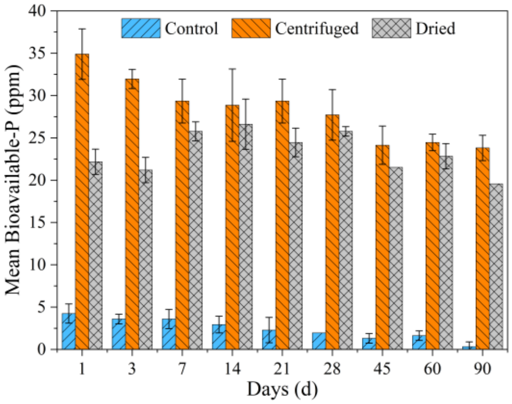

Fig. 6 Mean bioavailable-P (ppm) with different treatments among three months. Each error bar represents one standard deviation

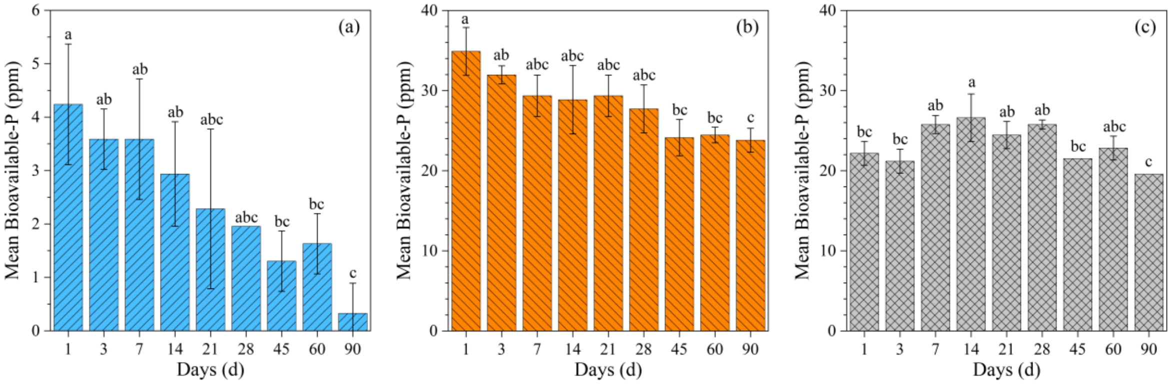

Fig. 7 Mean bioavailable-P (PPM) in soil of control (**a**), centrifuged (**b**) and dried (**c**) among three months. Different letters indicate differences between PPM levels that are significant at $P < 0.05$ (Tukey HSD). Each error bar represents one standard deviation

3.4. Decreasing kinetic of phosphorus bioavailability

Results of the coefficients of determination (r^2) and the standard error of estimate (SE) for kinetic equations, which were used to describe phosphorus bioavailability in the poor, alkaline and sandy soil are represented in **Table 4**. Firstly, the kinetic of control bioavailable-P can be well described by the first-order equation due to the high r^2 and low SE values and, secondly, by simple Elovich equation. Concerning the kinetic of bioavailable-P in the soil treated with centrifuged SSAD, the Elovich equation seems to be the best model for fitting these data due to the high r^2 (0.93). On the other hand, this model had a high SE (1.05). As well, the power equation successfully described this kinetic, with a high r^2 (0.91) and very low SE (0.04). Similar results were obtained in the work of Sinegani and co-workers [13]: in that study, potassium dihydrogen phosphate (KH_2PO_4) was added to sterile soil and the Elovich kinetic equation was the equation that better described the processes. As introduced in the previous paragraph, the most probable processes that could cause a decrease in $NaHCO_3$ extractable-P were precipitation and adsorption. Precipitation can be caused by Ca^{2+}, Fe^{3+} and Al^{3+} ions. From previous analyses on the sandy soil, it resulted that total limestone was 369 g/kg, active limestone was 10 g/kg and the exchangeable calcium was 950 ppm. With these high values of Ca^{2+} and a soil pH > 8, Ca^{2+} resulted the predominant cation and the soluble phosphorus can reasonably precipitate forming Ca phosphates [8,10]. As well the increase of pH in soil solution, for the principle of Le Chatelier, the formation of Ca phosphates is favored by the increasing of Ca^{2+} and P concentrations in soil solution. Many reactions could have occurred, but, at this soil pH, it is very probable that HPO_4^{2-} quickly reacted with calcium forming a sequence of products with decreasing solubility immediately after the addition of P to the soil [45]. Firstly, the dicalcium phosphate (slightly soluble) was formed followed by the tricalcium phosphate (very low soluble). The reactions are axpressed in equation (11):

$$HPO_4^{2-} \xrightarrow{CaCO_3} 2(CaHPO_4 \cdot 2H_2O) + CO_2 \uparrow \xrightarrow{CaCO_3} Ca_3(PO_4)_2 + CO_2 \uparrow + 5H_2O \tag{11}$$

The solubility of these compounds and, consequently, bioavailable-P, decrease as the phosphorus changes from HPO_4^{2-} ion to tricalcium phosphate. After that, the formation of more insoluble compounds could have continued forming (in order of decreasing solubility): oxyapatite $[3Ca_3(PO_4)_2]\cdot CaO$, hydroxyapatite $[3Ca_3(PO_4)_2]\cdot Ca(OH)_2$, carbonate apatite $[3Ca_3(PO_4)_2]\cdot CaCO_3$ and finally fluorapatite $[3Ca_3(PO4)_2]\cdot CaCO_3$ [45].

As introduced before, also cations of Fe and Al can precipitate with P forming Fe and Al phosphates such as variscite and strengite [48] (equations (12) and (13)):

$$Al^{3+} + H_2PO_4^-{}_{(aq)} + 2H_2O \rightleftarrows Al(PO_4) \cdot 2H_2O_{(S)} + 2H^+ \quad \text{variscite} \tag{12}$$

$$Fe^{3+} + H_2PO_4^-{}_{(aq)} + 2H_2O \rightleftarrows Fe(PO_4) \cdot 2H_2O_{(S)} + 2H^+ \quad \text{strengite} \tag{13}$$

These reactions are common in acidic soils with P additions due to the instability of Fe and Al cations at low pH, but in alkaline soils are rare. On the other hand, the addition of Fe salts (such as $FeSO_4 \cdot 7H_2O$) is adopted in WWTPs as a strategy to remove P from the water via precipitation and, hence, an accumulation of Fe phosphates in SS likely occurred [42,49,50]. In this way, the used SSADs were rich in terms of Fe phosphates and their addition to a moist alkaline soil could have caused the dissolution of Fe phosphates, enhancing P in the soil solution [50]. Huang and colleagues [42] found that after some days from SSADs application (chemically stabilized with $FeSO_4 \cdot 7H_2O$) to an alkaline soil, was observed an increase of bioavailable-P, due to P mineralization or mineral transformations. That could be another hypothesis explaining the enhancement of bioavailable-P after some days from the application of dried SSAD treatment. The other phenomenon that might have happened was the adsorption to Al and Fe oxides and hydroxides and the adsorption on the edges of alumino-silicate minerals. On the other hand, adsorption on oxides and hydroxides of Fe and Al and on alumino-silicate minerals tends to decrease with the increase of pH [48]. Differently, in a calcareous soil with a basic soil pH, rich in calcium carbonate it was highly possible that adsorption on calcite has occurred [51,52]. Regarding bioavailable-P kinetic with dried SSAD addition, none of the kinetic models proposed had a satisfactory fitting. On the other hand, similar results were obtained in the work of Sinegani and colleagues: in that work, the same models tested here resulted satisfied for sterilized soil with P addition [13]. Furthermore, in that work there was a treatment that was not satisfied by any of the tested models: after around 20 days from P addition to an unsterilized soil there was an increase in P-bioavailability. This increase was very similar to what happened in the soil treated with dried SSAD. In that case, the authors motivated the increasing of P with the release of organic acids (e.g. α-keto glucorunic acid [53]) by phosphate solubilizing microorganisms. These organic acids can chelate Ca, Fe and Al, causing an increase in the concentration of P in soil solution. This motivation can further explain the peak, but it is probably unrealistic for this case study due to the sterilization of soil combined with heat treatment of SSAD. In **Fig. 8** the experimental mean values of bioavailable-P compared with values of bioavailable-P applying different kinetic models are graphically represented. It is possible to note that, especially in centrifuged-treated soil, the P decreasing occurred in two stages: firstly, a rapid stage of loss of bioavailable-P and secondly, a gradual decreasing stage in which it seems that bioavailable-P reach the equilibrium. Many works reported that after an addition of P, the equilibrium was reached in different times: in laboratory conditions from only 15 seconds [51] to 60 days [54] and until 15 years in field conditions [55]. According to the literature, the same two different stages of P-sorption by soils observed in this work [13,32]. The first quick stage is attributed to adsorption (ligand exchange) and the second slow stage by precipitation into increasingly less soluble states as time increases [26]. Moreover, if P concentration is high (such as in centrifuged-treated soil), ligand exchange and surface precipitation can be considered as a continuum because of a rapid P precipitation [12,48,54]. These motivations can explain the bioavailable-P decreasing kinetic obtained in control and centrifuged-treated soil. Furthermore, the use of Elovich model to describe this kinetic is totally in line with previous motivations: in fact, the Elovich model is often used to describe soils reactions (such as PO_4^{3-} sorption and desorption) that could be described only by the combination of two or three first-order reactions [32,33]. Hence, the Elovich equation can describe the probable adsorptive/precipitate process that appeared in control and centrifuged treated soils. Finally, in a study with similar SSADs added to a similar soil it was suggested that the rate-determining step was the precipitation of Ca-phosphates [42].

Table 4 Coefficient of determination (r^2) and standard error of estimate (SE) for kinetic equations used to describe the bioavailable-P data

Treatment	First order		Second order		Simple Elovich		Power function	
	r^2	SE	r^2	SE	r^2	SE	r^2	SE
Control	0.91	0.25	0.73	0.49	0.90	0.42	0.64	0.50
Centrifuged	0.79	0.06	0.82	0.00	0.93	1.05	0.91	0.04
Dried	0.28	0.10	0.30	0.00	0.01	2.59	0.01	0.11

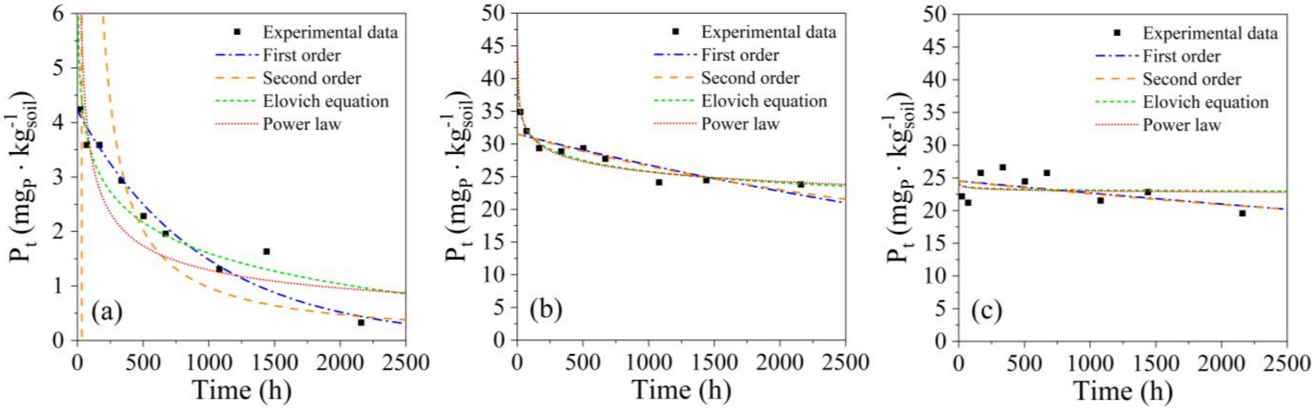

Fig. 8 Kinetic of bioavailable-P in soils over 2160 hours (90 days) in: (**a**) not treated soil, (**b**) treated with centrifuged SSAD soil and (**c**) treated with dried SSAD soil. Black points represent the mean values of experimental bioavailable-P, while colored lines represent the fitted kinetic models of bioavailable-P

4. Conclusion

In this experiment, phosphorus changings after the addition of SSADs to an alkaline soil were evaluated. Fractionation of P in SSADs revealed that most of it was in the inorganic form and the majority of this last was formed by NAIP, the most bioavailable fraction of P-Inorg. After the addition of SSADs to soil, it was seen the increment of P-Tot of around 100 ppm was supposed to be. The P-Tot content remained constant for all three months of the experiment. Differently, the bioavailable-P decreased from day 1 to day 90 in control and in soil treated with centrifuged SSAD. This decrease could be due principally to the precipitation of P with calcium: in fact, at alkaline pH and with a great presence of Ca^{2+} ions, the formation of sequential calcium phosphates, even less soluble along time, appeared to be the principal phenomenon. Furthermore, it was even possible that P adsorption occurred at a lesser extent. Due to the high pH of soil solution, it unlikely occurred on Al and Fe oxides and hydroxides and alumino-silicates since it was probably adsorbed on calcite surfaces. These explications are in line with other literature studies [8,10,13,45] and with some of the models tested (Elovich equation, power function). In particular, the Elovich model, due to the high r^2 and low SE, seems to best describe the adsorptive-precipitate process of bioavailable-P in control and centrifuged-treated soils. None of the models tested described satisfactorily the behavior of bioavailable-P in dried SSAD tested soil. In fact, on day 14 from treatment addition, there was an increment in P-bioavailability. The same behavior was also found in other works and could be caused by an anoxic environment, microorganisms, or organic matter. Future works will be directed on improving the techniques for phosphorus fractionation with the SMT method in order to reduce the “missing parts” of P. In this way, it will be possible to create specifics time course for all P fractions. Finally, it would be also important to deeply investigate on the behavior of dried SSADs, in order to unequivocally define which was the cause of the enhancing of bioavailable-P 14 days after SSAD addition. In conclusion, it was possible to assess the trend of bioavailable-P in a calcareous sandy soil fertilized with SSAD. This work paves the way for further studies related to kinetics of P-related phenomena. Indeed, they are not only crucial to design fertilizers and to optimize their efficiency, but also to reduce drawbacks resulting from unwise fertilization such as eutrophication.

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6. References

[1] J.B. Jones Jr., Plant Nutrition and Soil Fertility Manual, 2012. https://doi.org/10.1201/b11577.

[2] N. Gilbert, The disappearing nutrient: phosphate-based fertilizers have helped spur agricultural gains in the past century, but the world may soon run out of them. Natasha Gilbert investigates the potential phosphate crisis, Nature. 461 (2009) 716–719.

[3] G. Tóth, R.A. Guicharnaud, B. Tóth, T. Hermann, Phosphorus levels in croplands of the European Union with implications for P fertilizer use, Eur. J. Agron. 55 (2014) 42–52. https://doi.org/10.1016/j.eja.2013.12.008.

[4] P.E. Fixen, A.M. Johnston, World fertilizer nutrient reserves: A view to the future, J. Sci. Food Agric. 92 (2012) 1001–1005. https://doi.org/10.1002/jsfa.4532.

[5] L. Reijnders, Phosphorus resources, their depletion and conservation, a review, Resour. Conserv. Recycl. 93 (2014) 32–49. https://doi.org/10.1016/j.resconrec.2014.09.006.

[6] L. Li, H. XianJin, W. Hui, D. YuanHua, X. Sisi, Estimation of environmental costs of chemical fertilizer utilization in China., Acta Pedol. Sin. 46 (2009) 63–69. https://www.cabdirect.org/cabdirect/abstract/20093193564.

[7] G. Cristina, E. Camelin, T. Tommasi, D. Fino, M. Pugliese, Anaerobic digestates from sewage sludge used as fertilizer on a poor alkaline sandy soil and on a peat substrate: Effects on tomato plants growth and on soil properties, J. Environ. Manage. 269 (2020) 110767. https://doi.org/10.1016/j.jenvman.2020.110767.

[8] M. Fekri, N. Gorgin, L. Sadegh, Phosphorus desorption kinetics in two calcareous soils amended with P fertilizer and organic matter, Environ. Earth Sci. 64 (2011) 721–729. https://doi.org/10.1007/s12665-010-0892-9.

[9] J.L. Havlin, S.L. Tisdale, W.L. Nelson, J.D. Beaton, Soil fertility and fertilizers, Pearson Education India, 2016.

[10] B.K. Yadav, J. Panwar, S.C. Yadav, S.I. Meena, K.I. Patel, M. Patel, A. Sayeed, Bioavailability of Soil P for Plant Nutrition, 10 (2012). https://doi.org/10.1007/978-94-007-4500-1.

[11] J.E. Yang, C.A. Jones, H.J. Kim, J.S. Jacobsen, Soil inorganic phosphorus fractions and Olsen-P in phosphorus-responsive calcareous soils: Effects of fertilizer amount and incubation time, Commun. Soil Sci. Plant Anal. 33 (2002) 855–871. https://doi.org/10.1081/CSS-120003071.

[12] E. Afif, A. Matar, J. Torrent, Availability of Phosphate Applied to Calcareous Soils of West Asia and North Africa, Soil Sci. Soc. Am. J. 57 (1993) 756–760. https://doi.org/10.2136/sssaj1993.03615995005700030022x.

[13] A.A. Sinegani, S. Sedri, Effects of sterilization and temperature on the decrease kinetic of phosphorus bioavailability in two different soil types, J. Soil Sci. Plant Nutr. 11 (2011) 109–122. https://doi.org/10.4067/S0718-95162011000200010.

[14] V. Ruban, J.F. López-Sánchez, P. Pardo, G. Rauret, H. Muntau, P. Quevauviller, Harmonized protocol and certified reference material for the determination of extractable contents of phosphorus in freshwater sediments - A synthesis of recent works, Anal. Bioanal. Chem. 370 (2001) 224–228. https://doi.org/10.1007/s002160100753.

[15] V. Ruban, J.F. López-Sánchez, P. Pardo, G. Rauret, H. Muntau, P. Quevauviller, Selection and evaluation of sequential extraction procedures for the determination of phosphorus forms in lake sediment, J. Environ. Monit. 1 (1999) 51–56. https://doi.org/10.1039/a807778i.

[16] C. Wang, Y. Zhang, H. Li, R.J. Morrison, Sequential extraction procedures for the determination of phosphorus forms in sediment, Limnology. 14 (2013) 147–157. https://doi.org/10.1007/s10201-012-0397-1.

[17] A. Moore Jr, K.R. Reddy, M.J. Hedley, J.W.B. Stewart, Bs. Chauhan, Role of Eh and pH on phosphorus geochemistry in sediments of Lake Okeechobee, Florida, J. Environ. Qual. 46 (1994) 970–976.

[18] J.D.H. Williams, J.K. Syers, T.W. Walker, Fractionation of Soil Inorganic Phosphate, Soil Sci. Soc. Am. J. 31 (1967) 736–739.

[19] M.J. Hedley, J.W.B. Stewart, Bs. Chauhan, Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations, Soil Sci. Soc. Am. J. 46 (1982) 970–976.

[20] M. García-Albacete, A. Martín, M.C. Cartagena, Fractionation of phosphorus biowastes: Characterisation and environmental risk, Waste Manag. 32 (2012) 1061–1068. https://doi.org/10.1016/j.wasman.2012.02.003.

[21] P. Pardo, G. Rauret, J.F. López-Sánchez, Shortened screening method for phosphorus fractionation in sediments: A complementary approach to the standards, measurements and testing harmonised protocol, Anal. Chim. Acta. 508 (2004) 201–206. https://doi.org/10.1016/j.aca.2003.11.005.

[22] J.J. González Medeiros, B. Pérez Cid, E. Fernández Gómez, Analytical phosphorus fractionation in sewage sludge and sediment samples, Anal. Bioanal. Chem. 381 (2005) 873–878. https://doi.org/10.1007/s00216-004-2989-z.

[23] W. Zhang, X. Jin, Y. Ding, X. Zhu, N. Rong, J. Li, B. Shan, Composition of phosphorus in wetland soils determined by SMT and solution 31P-NMR analyses, Environ. Sci. Pollut. Res. 23 (2016) 9046–9053. https://doi.org/10.1007/s11356-015-5974-5.

[24] C. Xie, J. Tang, J. Zhao, D. Wu, X. Xu, Comparison of phosphorus fractions and alkaline phosphatase activity in sludge, soils, and sediments, J. Soils Sediments. 11 (2011) 1432–1439. https://doi.org/10.1007/s11368-011-0429-1.

[25] S.R. Olsen, C. V Cole, F. Watandbe, L. Dean, Estimation of Available Phosphorus in Soil by Extraction with sodium Bicarbonate, J. Chem. Inf. Model. 53 (1954) 1689–1699. https://doi.org/10.1017/CBO9781107415324.004.

[26] H.L. Bohn, G.A. O'Connor, B.L. McNeal, Soil Chemistry, 3rd ed., 2001.

[27] A. Hosseinpur, H. Pashamokhtari, Impact of treated sewage sludge application on phosphorus release kinetics in some calcareous soils, Environ. Geol. 55 (2008) 1015–1021. https://doi.org/10.1007/s00254-007-1050-x.

[28] G. Cristina, E. Camelin, C. Ottone, S. Fraterrigo, L. Jorquera, M. Castro, D. Fino, M. Cristina, T. Tommasi, Recovery of humic acids from anaerobic sewage sludge: extraction , characterization and encapsulation in alginate beads, Int. J. Biol. Macromol. 164 (2020) 277–285. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.07.097.

[29] G. Cristina, E. Camelin, M. Pugliese, T. Tommasi, D. Fino, Evaluation of anaerobic digestates from sewage sludge as a potential solution for improvement of soil fertility, Waste Manag. 99 (2019) 122–134. https://doi.org/10.1016/j.wasman.2019.08.018.

[30] J.D.H. Williams, T. Mayer, J.O. Nriagu, Extractability of phosphorus from phosphate minerals common in soils and sediments, Soil Sci. Soc. Am. J. 44 (1980) 462–465.

[31] W.E. Federation, A.P.H. Association, Standard methods for the examination of water and wastewater, Am. Public Heal. Assoc. Washington, DC, USA. (2005).

[32] D.L. Sparks, Kinetics of Soil Chemical Processes-Academic Press, 1989.

[33] S.H. Chien, W.R. Clayton, Application of Elovich Equation to the Kinetics of Phosphate Release and Sorption in Soils, Soil Sci. Soc. Am. J. 44 (1980) 265–268. https://doi.org/10.2136/sssaj1980.03615995004400020013x.

[34] M. Islas-Espinoza, L. Solís-Mejía, M. V. Esteller, Phosphorus release kinetics in a soil amended with biosolids and vermicompost, Environ. Earth Sci. 71 (2014) 1441–1451. https://doi.org/10.1007/s12665-013-2549-y.

[35] M. Karamesouti, D. Gasparatos, Sustainable Management of Soil Phosphorus in a Changing World, in: Adapt. Soil Manag. From Theory to Pract., Springer Singapore, Singapore, 2017: pp. 189–214. https://doi.org/10.1007/978-981-10-3638-5_9.

[36] C. Wang, Q.W. Liu, Y. Zhi, L. Cheng, N. Wang, C.D. Li, Y.X. Mao, Contents and Forms of Phosphorous in the Municipal Sewage Sludge of China, Huanjing Kexue/Environmental Sci. 40 (2019) 1922–1930. https://doi.org/10.13227/j.hjkx.201809078.

[37] C. Nobile, D. Houben, E. Michel, S. Firmin, H. Lambers, E. Kandeler, M.P. Faucon, Phosphorus-acquisition strategies of canola, wheat and barley in soil amended with sewage sludges, Sci. Rep. 9 (2019) 1–11. https://doi.org/10.1038/s41598-019-51204-x.

[38] R. Li, J. Yin, W. Wang, Y. Li, Z. Zhang, Transformation of phosphorus during drying and roasting of sewage sludge, Waste Manag. 34 (2014) 1211–1216. https://doi.org/10.1016/j.wasman.2014.03.022.

[39] M. Tibbert, M.T.E. Smith, B.J. Cade-Menun, Phosphorus dynamics under Lolium perenne in a sandy loam soil after a single application of three different sewage sludge types from the same treatment stream, in: Proc. 2nd Int. Symp. Phosphorus Dyn. Soil-Plant Contin. Perth, West Aust., 2003: pp. 28–29.

[40] Arpa Veneto, Capitolo 13 - Agrelan: un sistema di interpretazione dell'analisi del terreno, in: L'interpretazione Delle Anal. Del Terreno - Strum. per La Sostenibilità Ambient., Padova, 2007: pp. 62–67.

[41] P. Pardo, J.F. López-Sánchez, G. Rauret, Relationships between phosphorus fractionation and major components in sediments using the SMT harmonised extraction procedure, Anal. Bioanal. Chem. 376 (2003) 248–254. https://doi.org/10.1007/s00216-003-1897-y.

[42] X.L. Huang, Y. Chen, M. Shenker, Dynamics of phosphorus phytoavailability in soil amended with stabilized sewage sludge materials, Geoderma. 170 (2012) 144–153. https://doi.org/10.1016/j.geoderma.2011.11.025.

[43] F. Bibi, I. Saleem, S. Javid, S. Ehsan, S. Danish, I. Ahmad, Phosphorus release kinetics of applied phosphate is influenced by time and organic sources in clay loam and sandy clay loam soils, Soil Environ. 37 (2018) 136–142. https://doi.org/10.25252/SE/18/20973.

[44] S. Javid, D.L. Rowell, A laboratory study of the effect of time and temperature on the decline in Olsen P following phosphate addition to calcareous soils, Soil Use Manag. 18 (2002) 127–134. https://doi.org/10.1079/SUM2002116.

[45] R.. Weil, N.C. Brady, Soil Phosphorus and Potassium, in: Nat. Prop. Soils, 15th ed., 2017: pp. 643–695.

[46] F.J. Stevenson, Humus chemistry: genesis, composition, reactions, John Wiley & Sons, 1994.

[47] C.N. Guppy, N.W. Menzies, P.W. Moody, F.P.C. Blamey, Competitive sorption reactions between phosphorus and organic matter in soil: A review, Aust. J. Soil Res. 43 (2005) 189–202. https://doi.org/10.1071/SR04049.

[48] C.J. Penn, J.J. Camberato, A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants, Agric. 9 (2019) 1–18. https://doi.org/10.3390/agriculture9060120.

[49] E. Epstein, Land application of sewage sludge and biosolids, LEWIS PUBLISHERS, 2001.

[50] X.-L. Huang, Y. Chen, M. Shenker, Solid Phosphorus Phase in Aluminum- and Iron-Treated Biosolids, J. Environ. Qual. 36 (2007) 549–556. https://doi.org/10.2134/jeq2006.0155.

[51] S. Kuo, E.G. Loxse, Division s-2—soil chemistry, (1972).

[52] Griffin R.A., Jurinak J.J., The Interaction of Phosphate with Calcite, Soil Sci. Soc. Am. J. (1973) 847–850.

[53] M.R. Motsara, P.B. Bhattacharyya, B. Srivastava, Biofertilizers their description and characteristics, Biofertiliser Technol. Mark. Usage. A Source Book-Cum-Glossary, Fertil. Dev. Consult. Organ. New Delhi. (1995) 9–18.

[54] Y.S.R. Chen, J.N. Butler, W. Stumm, Kinetic Study of Phosphate Reaction with Aluminum Oxide and Kaolinite, Environ. Sci. Technol. 7 (1973) 327–332. https://doi.org/10.1021/es60076a007.

[55] Y. Ma, J. Li, X. Li, X. Tang, Y. Liang, S. Huang, B. Wang, H. Liu, X. Yang, Phosphorus accumulation and depletion in soils in wheat-maize cropping systems: Modeling and validation, F. Crop. Res. 110 (2009) 207–212. https://doi.org/10.1016/j.fcr.2008.08.007.