Research Article

A novel $FePO_4$ nanosized fertilizer is as effective as triple superphosphate in sustaining the growth of cucumber plants

Andrea CIURLI[1,5], Laura GIAGNONI[2], Davide SEGA[1], Roberta PASTORELLI[3], Zeno VARANINI[1,*], Giancarlo RENELLA[4] and Anita ZAMBONI[1]

[1]*Biotechnology Department, University of Verona, 37134 Verona (Italy)*
[2]*Department of Civil Engineering, Architecture, Environmental and Mathematics (DICATAM), University of Brescia, via Branze 43, Italy c Research Centre for Agriculture and Environment, 25123 Brescia (Italy)*
[3]*Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, I-50125 Florence (Italy)*
[4]*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, 35020 Legnaro (Italy)*
[5]*Current address: Department of Agricultural and Food Sciences (DISTAL), University of Bologna. Via G. Fanin 40, 40147 Bologna (Italy)*

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ABSTRACT

The behaviour of nanofertilizers (NF) in plant-soil systems can differ from that of conventional chemical fertilizers due to their peculiar chemical-physical properties. Their effectiveness is still poorly understood. Here, in a plant-soil microcosm, we evaluated the P fertilization potential of a novel nanosized $FePO_4$ NF. We tested the efficacy of a $FePO_4$ NF in sustaining the growth of cucumber plants in a pot experiment, compared to a conventional triple superphosphate (TSP) fertilizer. Plants were grown for 28 days on a P deficient soil and determinations were made of growth parameters, nutrient concentrations in plant tissues, P availability in soil, activity of enzymes involved in C, N, P and S mineralization and the structure of the soil microbial communities. Results showed no significant differences in dry weight, leaf area, SPAD index or root growth between NF and TSP fertilized plants. Conversely, P availability in soil and P content in plant tissues at the end of the experiment were significantly higher after TSP compared to NF fertilization, whereas no major differences were observed for other nutrients. Among the measured soil enzyme activities, similar values for acid phosphatase, β-glucosidase and arylsulfatase activities were found between NF- and TSP-treated soils, the alkaline phosphatase activity presented higher values in TSP- than in NF-fertilized soil, while the protease activity showed higher values in NF- than in TSP-fertilized soils. Microbial community structure of NF- and TSP-fertilized soils showed significant differences for archaeal, bacterial, and fungal communities, although the microbial community profiles generally clustered closer to each other in all treatments. We concluded that the $FePO_4$ NF tested can be an efficient alternative to conventional TSP fertilizers.

Keywords: conceptual model, enzyme activity, nanoparticles, P availability, P nutrition, plant-soil system

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INTRODUCTION

The chemical fertilization of crops is essential to meet the nutritional needs of the burgeoning global population, which is projected to reach 11.2 billion by 2100 (Adam, 2021), and to guarantee adequate crop yields and incomes to farmers. Fertilizers applied to crops result in a variable nutrient use efficiency (NUE), which is low in conventional NPK fertilizers, and after fifty years of a downward trend, the average rate of applied P in Italian agricultural soils is 26 kg ha^{-1} (ISPRA, 2020). Plant phosphorus (P) uptake occurs as HPO_4^{2-} or $H_2PO_4^-$ anions, the concentration in soil solution of which is in the order of micromoles (Bieleski,

*Corresponding author. E-mail: zeno.varanini@univr.it.

1973). After fertilization, conventional P fertilizers (*e.g.* triple superphosphate, TSP) undergo the linear release of highly soluble P forms into the soil solution, which can leach into the groundwater and cause acidification or be immobilized by soil microorganisms (Chen *et al.*, 2018). When conventional P fertilizers dissolve in a soil solution of acidic soils, they induce dissolution of soil solid phases with the release of cations that can lead to low solubility P forms whereas, in alkaline soils, P phytoavailability is limited by the formation of sparingly soluble Ca and Mg phosphates. For these reasons, P remains the least available nutrient and the one that chiefly limits crop productivity, with a NUE ranging from 20% to 30% (López-Arredondo *et al.*, 2014) or even as low as 10% (Baligar *et al.*, 2001), regardless of the total P content. A wide range of physiological and metabolic responses can be triggered by limited P availability for plants, such as reduced leaf expansion (Hawkesford *et al.*, 2012), lower photosynthetic activity (Carstensen *et al.*, 2018), and modified root architecture displaying increased lateral roots with a significant impact on crop production (López-Bucio *et al.*, 2002). Any improvement of P use efficiency by crop plants may therefore greatly mitigate the environmental impact of agriculture and increase the sustainability for farmers. To improve PUE, the fertilizer industry has evolved technologies to devise products that release nutrients more slowly than TSP, whether by physico-chemical or microbial decomposition processes, mainly by coating fertilizer granules with water-insoluble films or pelleted multilayer structures that retard solubilization in the soil solution and thereby prevent leaching and run-off (Chen *et al.*, 2018). Organic fertilizers ensure the gradual release of nutrients, without additional synthetic by-products, and have a higher crop NUE, but they are mainly used in organic farming and result in lower crop yields.

Nanotechnology is among the most promising technologies being applied in agriculture and various other fields, based on the peculiar surface properties of nanoparticles (Chhipa, 2019). Nanofertilizers (NFs) are formulations that involve the synthesis of materials measuring between 1 and 100 nm. However, more than a decade after the first formulations were proposed (Wang *et al.*, 2012; Sarkar *et al.*, 2014), synthesis and the use of NFs are still in their infancy. Nanofertilizers can be produced through physical (top-down approach), chemical (bottom-up approach), or biological (green synthesis) technologies (Dimkpa and Bindraban, 2018; Nongbet *et al.*, 2022). Since the top-down approach results in poor control of nanoparticle (NP) size, and the biological synthesis is still limited by the small scale and high costs (Prasad *et al.*, 2016), production of NFs occurs mainly through bottom-up synthesis; this technique ensures the production of NF with controlled sizes and physico-chemical properties, at low cost (Zulfiqar *et al.*, 2019). Owing to their size and surface properties, NFs have been reported to release nutrients more gradually than the bulk equivalent (DeRosa *et al.*, 2010), thereby reducing nutrient losses from soils, enhancing plant NUE and increasing crop yield (Dewdar *et al.*, 2018; Kandil *et al.*, 2020). Promising P nanofertilizers have recently been created using biodegradable polymer nanocomposites (Sigmon *et al.*, 2021), and chitosan and Zn-oxide nanoparticle-enhanced tripolyphosphate (Dimkpa *et al.*, 2023). It has also been reported that NFs could promote plant growth in a similar way to biostimulants (Shylaja *et al.*, 2022; Wang *et al.*, 2023). Notwithstanding their potential, NFs are still little used because their effects vary on different crops (Lin and Xing, 2007; Kalia *et al.*, 2019; Surendhiran *et al.*, 2020).

In this work, we tested the fertilization potential of a novel nanosized $FePO_4$ NF produced with a protocol developed by Sega *et al.* (2019). $FePO_4$ NFs are a very promising material since P and Fe are two essential mineral nutrients able to limit the plant yield (Broadley *et al.*, 2012; Hawkesford *et al.*, 2012). Furthermore, it is known that plant strategies for Fe and P mobilization share several mechanisms, such as proton extrusion and the secretion of carboxylic acids and phenolic compounds (Watt and Evans, 1999; Tomasi *et al.*, 2009). This NF was shown to release P and ensure P bioavailability at slower rates than conventional TSP and does not have any negative effects on soil microbial communities, nor on soil microbial activity (Ciurli *et al.*, 2022). The P fertilization potential of the novel NF was evaluated in a plant cultivation trial on soil characterized by limited P availability, due to its sandy texture and acidic pH value.

MATERIALS AND METHODS

$FePO_4$ NF synthesis and characterization

The NF was made up of citrate-capped $FePO_4$ nanoparticles (NPs) synthesized as described by Sega *et al.* (2019). NP size distribution was determined through DLS analysis with a Malvern Zetasizer (ZS instrument) operating with a He-Ne laser at 633 nm. Samples for DLS analysis were diluted 1:20 in deionized water and analysed by measuring 173° backscatter (Fig. S1, see Supplementary Material for Fig. S1). The analysis showed a peak at 91 nm, thereby confirming that the NF was of dimensions compatible with nano-sized materials (< 100 nm). The Fe and P quantification were carried out according to (Stookey,

1970) and (Murphy and Riley, 1962), respectively. The concentration of Fe and P was equal to 0.15 and 0.12 M respectively, and the Fe/P ratio was equal to 1.25.

Plant growth, fertilization, and chemical analyses

A Eutric Cambisol sandy loam soil was sampled at an abandoned crop site located in Romola (Florence, Italy). Table I summarizes the main chemical characteristics of the soil.

TABLE I

Main chemical characteristics of the soil

Property	Value
Organic matter % (w/w)	2.95
Total N % (w/w)	0.11
Available P (Olsen) (mg kg^{-1})	3.00
Exchangeable Ca (mg kg^{-1})	1860
Exchangeable K (mg kg^{-1})	69
Exchangeable Mg (mg kg^{-1})	406
Oxalate-extractable Fe (g kg^{-1})	2.95
pH (H_2O)	5.40

Cucumber seeds (*Cucumis sativus* viridis, F1 hybrid) were germinated in the dark at 25°C on paper soaked with 1 mM $CaSO_4$. One seedling was transferred to a 0.5 dm^3 pot, making a total of 5 pots per treatment, filled with 375 g of dry soil sieved at 5 mm. Plants were grown for 28 days in a 16/8 light photoperiod, at 25 ± 1 °C, 50% ± 5 air humidity, light irradiance of 200 µmol m^{-2} s^{-1} as PPFD (Photosynthetic Photon Flux Density). Plants were fertilized four times on days 1, 7, 14 and 21 with a solution of $Ca(NO_3)_2$ and plus NF or TSP to reach final rates of 80 mg kg^{-1} dry soil of nitric-N and 34 mg kg^{-1} of P. The -P treatment received the same volumes of $Ca(NO_3)_2$ alone. The experiment was repeated three times independently with 5 plants and soils for each experiment.

The leaf area and the soil-plant analysis development (SPAD) index were measured after 14, 21 and 28 days of growth by calculating the average value of five measurements per leaf with a SPAD-502 Plus Chlorophyll meter® (Konica Minolta) for each plant, always taken on the first leaf. In the case of leaf area, images were taken of the plants every week and analysed with ImageJ® free software (http://imagej.nih.gov/ij/). Leaf area was calculated by scaling the measurements to a 1-cm marker in each picture, placed beforehand on each pot so that they would reach the highest point of the canopy of each plant. At the end of the experiment, the whole root apparatus was washed with water several times to remove residue soil. Roots were then washed 5 times with deionized water (18.2 MW·cm at 25 °C). The root images were acquired with an EpsonV700 perfection scanner. The results were then analysed with WinRHIZO™ software 2015a Pro version (Regent Instruments Inc.) using the 'root morphology' mode. After the determination of dry weight, elemental analysis was carried out on shoot and root samples.

Plant elemental analysis was conducted on milled tissue digested with 350 µl of ultrapure grade 69% HNO_3 (Romil LTD) using a 3-mL TFM microsampling insert (Milestone), and three inserts were placed inside a TFM 100-mL vessel with 11 mL Milli-Q water and 1 mL of ultra-pure grade 30% H_2O_2 (Romil LTD). Plant tissue digestion was performed at 180 °C for 20 min in a StartD (Milestone) microwave digester. Digested samples were diluted with ultra-pure grade water and acidified with 2% of HNO_3 prior to ICP-MS elemental analysis (7500ce, Agilent technologies). Elemental concentrations were determined against a calibration curve based on a certified multielement standard solution (Romil LTD), and the efficiency of the elemental analysis was checked using NIST standard reference material (SRM 1515).

The P availability in soil was determined in accordance with Olsen and Sommers (Olsen and Sommers, 1983), using 0.5 M $NaHCO_3$ as extractant and solphomolybdic reagent to develop the coloured complex with phosphate-P. Absorbance values were measured at 720 nm by Agilent Cary60 spectrophotometer, and the P concentration was determined using a calibration standard curve.

Analysis of the soil microbial community

Analysis of the microbial community was performed on each soil sample prior to cucumber transplant (T0) and at the end of the plant growth period. Genomic DNA was extracted from each soil sample after plant growth using DNeasy® PowerLyzer® PowerSoil® Kit (QIAGEN) according to the manufacturer's

instructions. Soils were homogenized using a FastPrep-24™ at speed of 6 m s^{-1} for 40 s, and the PCR amplifications were performed using a T100 Thermal Cycler (Bio-Rad) under the following reaction conditions: 95 °C for 3 min, denaturation for 95 °C for 30 sec, annealing for 30 sec (55 °C for bacteria and archaea and 48 °C for fungi), and extension to 72 °C for 45 sec (34 cycles), followed by 5 min at 72 °C. Bacterial 16S rDNA amplicons were obtained using the GC986f – UNI1401r primer set (Felske *et al.*, 1998) and DGGE was carried out in a 6% polyacrylamide gel with a 45-65% denaturing gradient. The fungal 18S rDNA amplicons were obtained using the EF390 – GCFR1 primer pair (Vainio and Hantula, 2000) in a 8% polyacrylamide gel with a 38-75% denaturing gradient. The quality of PCR amplicons was checked on an agarose gel and yields were estimated by comparing amplified DNA to Low DNA mass ladder (Invitrogen) using the Chemidoc system (Bio-Rad). The DGGE analysis was performed using a polyacrylamide gel (acrylamide/bis 37.5:1) with a linear denaturing gradient obtained with a 100% denaturant solution consisting of 40% v/v formamide and 7M urea, in a DCodeTM System (Bio-Rad), stained with SYBR®Gold (Invitrogen), and fingerprint images were digitized under UV using the Chemidoc system.

Soil enzyme activity

Analysis of the enzyme activities involved in C, N and P biogeochemical cycles was performed on the T0 soil and at the end of the plant growth period on each treated soil sample. Enzyme activities were determined on the T0, and at the end of the plant growth period. Acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969). Arylsufatase activity was determined according to Tabatabai and Bremner (1970), β-glucosidase activity was determined according to Tabatabai (1983). Protease activity was determined by the release of tyrosine after incubation with sodium caseinate (mg Tyr g^{-1} soil) (Ladd and Butler, 1972).

Data analysis

Results were presented as mean ± standard deviation and were analysed through One-way ANOVA followed by post hoc t-test and Tukey test using the GraphPad Prism 7 (GraphPad Software).

The DGGE band migration distance and intensity of quantitative (non-binary) matrices were evaluated by GelCompar II v.46 software (Applied Maths). The banding patterns of each DGGE were extracted as band-intensity matching tables and imported into Past software version 3.22 (Hammer *et al.*, 2001) for multivariate analysis. Non-metric multidimensional scaling (nMDS) was used to display differences of DGGE profiles in two-dimensions; the accuracy of the nMDS plots was determined by calculating a 2D stress value. One-way analysis of similarity (ANOSIM) was performed to determine significant differences within different groups of data (treatments) (Ramette, 2007; Pastorelli *et al.*, 2020). The nMDS and ANOSIM were carried out using the Bray-Curtis distance measure and with 9999 permutational tests.

RESULTS

Plant growth and physiological parameters, and soil P availability

Measured plant growth parameters displayed no significant differences in shoot and root dry weight (Fig. 1a), leaf area, SPAD index and root growth between NF- and TSP-fertilized plants (Fig. 2).

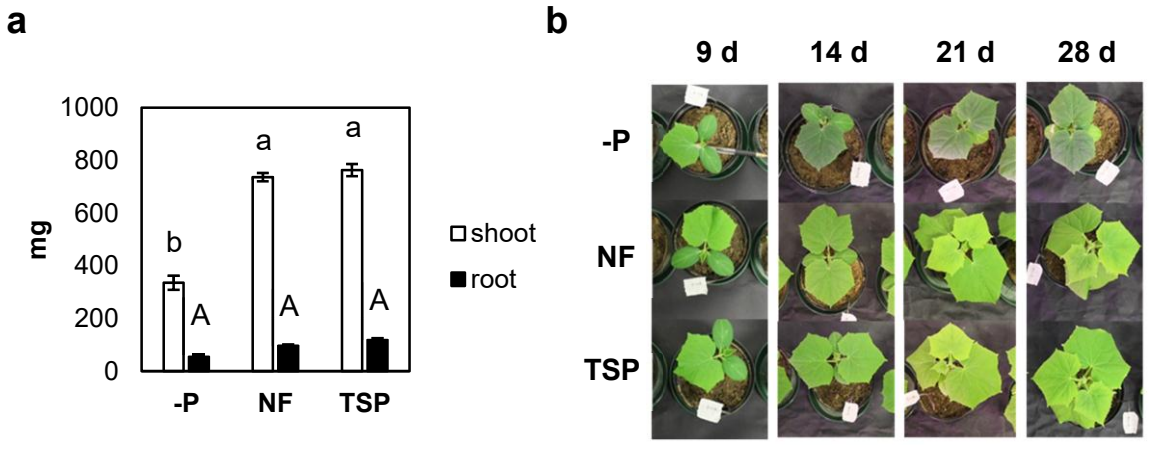


Fig. 1. Growth of cucumber plants. (a) Shoot and root dry weight determined at the end of the experiment (28 days) of P-deficient, NF-treated, and TSP-treated plants. Data are expressed as the mean ± S.E. of three independent experiments with five plants each (One-way ANOVA with Tukey's test, n= 15 plants, P< 0.05, different letters indicate significant differences between the analysed conditions, lowercase letter: shoot samples; uppercase letter: root samples). (b) Pictures of plant shoots of -P, NF- and TSP-treated plants after 9, 14, 21 and 28 days.

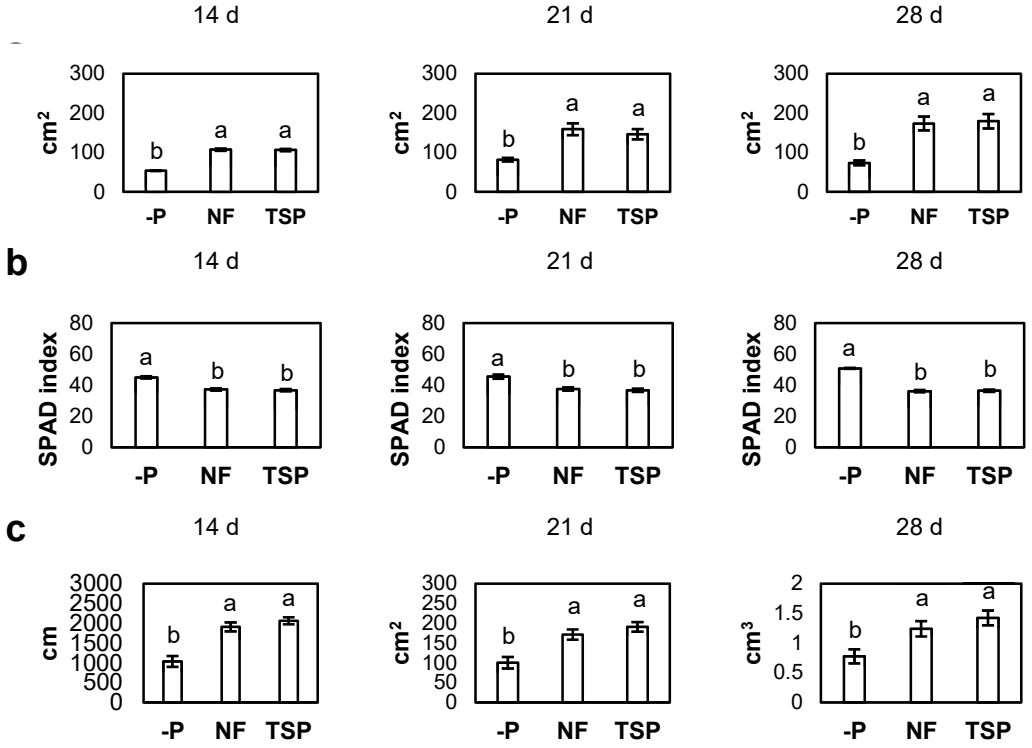


Fig. 2 Leaf and root parameters of cucumber plants and P and Fe content in plant tissues. (a) Leaves area and (b) SPAD index measured after 14, 21 and 28 days of growth. (c) root length, root surface area and root volume determined at 28 days. Data are expressed as the mean ± S.E. of three independent experiments with five plants each (One-way ANOVA with Tukey's test, P< 0.05, different letters indicate significant differences between the analysed conditions, n = 15 plants for leaf parameters, n = 10 plants for root parameters). -P: P-deficient plants; NF: NF-treated plants; TSP: TSP-treated plants.

Both treatments proved able to increase the plant biomass and prevent P-deficiency symptoms, as confirmed by the lower SPAD index values (Fig. 2b). At root level, no significant differences in length, surface and volume were recorded between plants supplied with NFs and TSP, whereas plant root parameters were almost double those of unfertilized plants (Fig. 2c).

The P concentration in shoot and root tissue was around 20% higher in TSP- than NF-treated plants, while plants grown on -P soil showed significantly lower P concentrations in both roots and shoots compared to those grown on NF- and TSP-fertilized soils (Table II).

TABLE II

Elements contained in shoot and root tissues of plants grown on control soils (-P) and in soils fertilized with NF and TSP determined after 28 days of growth

	Shoot		
	-P	NF	TSP
P (mg gDW^{-1})	0.95 ± 0.12 c[a)]	3.60 ± 0.21 b	4.47 ± 0.22 a
K (mg gDW^{-1})	25.36 ± 2.59 a	27.64 ± 2.57 a	26.34 ± 1.78 a
Ca (mg gDW^{-1})	18.46 ± 1.69 a	14.39 ± 1.68 a	15.32 ± 1.75 a
Mg (mg gDW^{-1})	5.72 ± 0.26 a	4.91 ± 0.38 a	5.62 ± 0.42 a
Fe (µg gDW^{-1})	202.32 ± 20.45 a	176.94 ± 20.59 a	161.13 ± 25.45 a
Mn (µg gDW^{-1})	78.80 ± 4.77 a	55.18 ± 3.28 b	63.83 ± 3.22 b
Zn (µg gDW^{-1})	74.54 ± 7.27 a	63.87 ± 5.33 a	71.49 ± 6.30 a
Cu (µg gDW^{-1})	11.55 ± 0.69 a	7.74 ± 0.74 b	8.36 ± 0.75 b
	Root		

	-P	NF	TSP
P (mg gDW^{-1})	1.19 ± 0.09 c	3.25 ± 0.14 b	4.05 ± 0.26 a
K (mg gDW^{-1})	36.25 ± 1.85 b	42.20 ± 1.26 a	40.57 ± 1.32 ab
Ca (mg gDW^{-1})	7.10 ± 0.19 a	6.68 ± 0.22 a	6.65 ± 0.19 a
Mg (mg gDW^{-1})	3.81 ± 0.18 a	3.14 ± 0.11 b	3.63 ± 0.12 a
Fe (µg gDW^{-1})	1996.70 ± 167.94 a	1359.92 ± 95.85 b	1508.83 ± 158.15 ab
Mn (µg gDW^{-1})	144.76 ± 10.91 a	81.01 ± 6.60 b	100.55 ± 4.88 b
Zn (µg gDW^{-1})	127.46 ± 7.82 a	108.57 ± 10.31 a	131.36 ± 12.62 a
Cu (µg gDW^{-1})	19.15 ± 1.59 a	17.62 ± 1.52 a	19.81± 1.29 a

[a)]Data are expressed as the mean ± S.E. of three independent experiments with five plants each (One-way ANOVA with Tukey's test, $P < 0.05$, different letters indicate significant differences between the analysed conditions, $n = 15$ plants).

Plants grown on P deficient soils displayed higher Mn and Cu and lower K concentrations in shoots (Table II). In P-fertilized plants, lower concentrations of Mg and Fe were found in roots of NF- than TSP-fertilized plants (Table II). The P-Olsen in soil at the end of the plant growth period showed significantly higher values in TSP- than in NF-fertilized soils, and significantly higher values in both TSP- and NF-fertilized soils than in -P soil (Fig. 3).

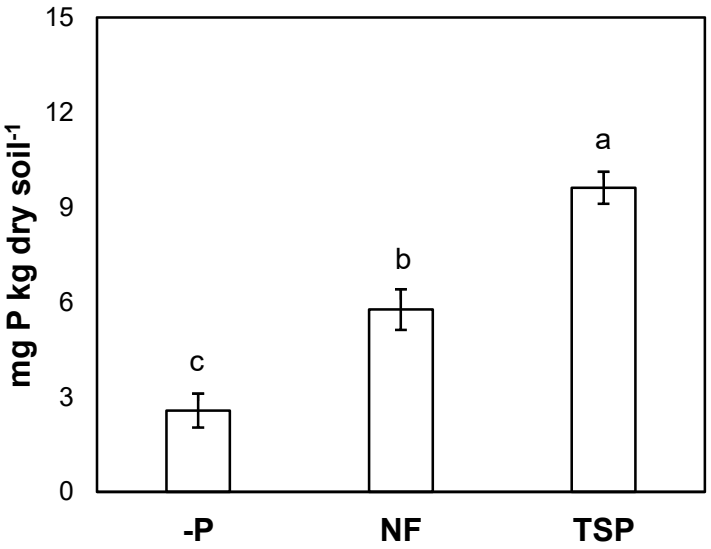
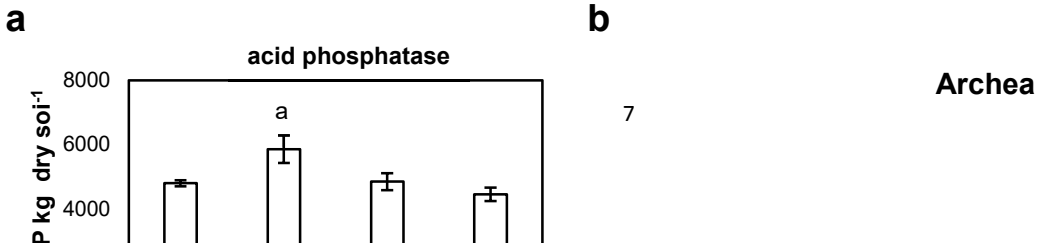
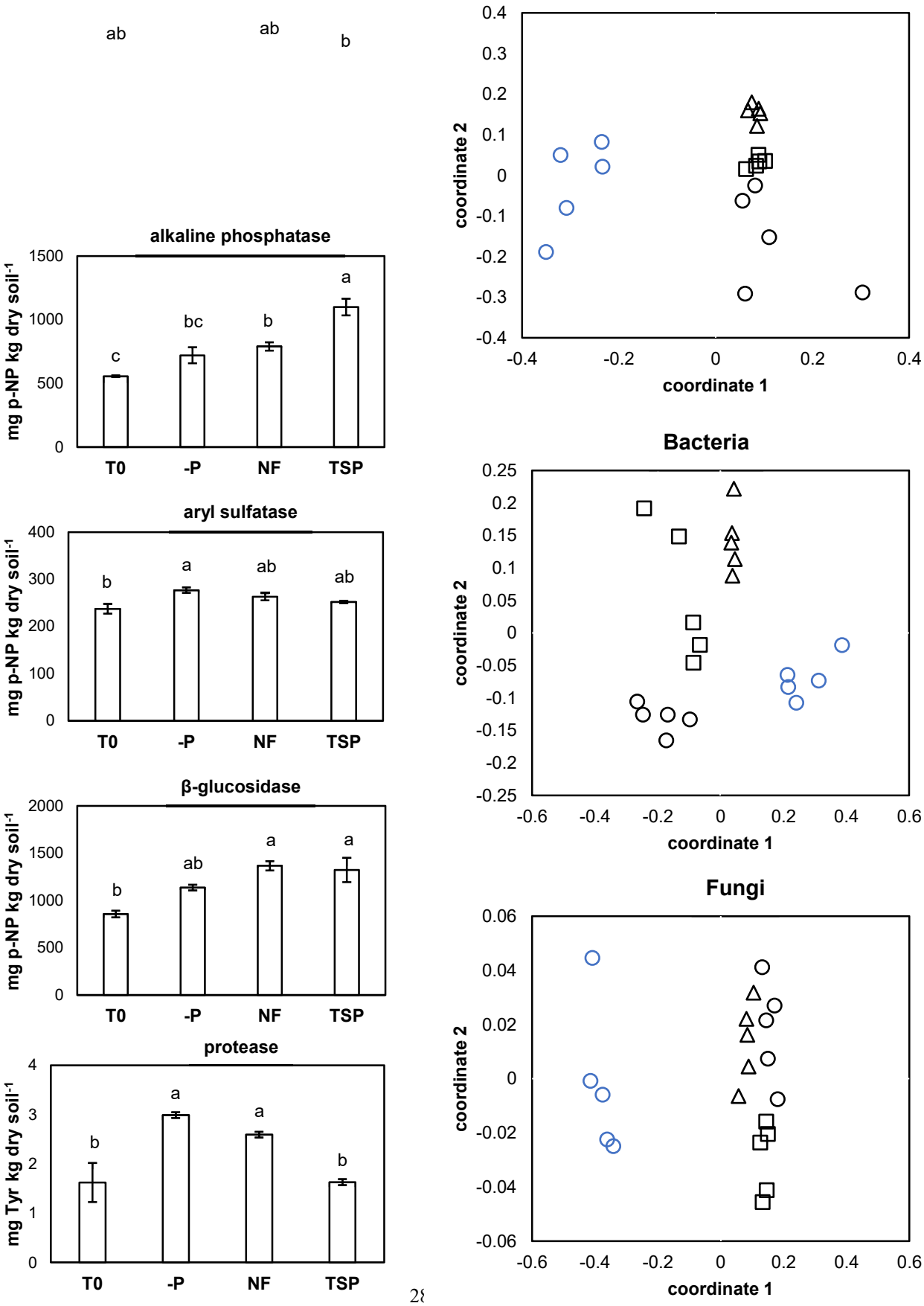


Fig. 3 P availability measured in rhizosphere soil after 28 days. Data are expressed as mean ± S.E. of three independent experiments (soils of three pots for each experiment, One-way ANOVA with Tukey's test, n= 9, P< 0.05, different letters indicate significant differences between the analysed conditions). -P: P-deficient plants; NF: NF-treated plants; TSP: TSP-treated plants.

Soil enzyme activities and microbial community structure

Acid phosphatase activity presented significantly higher values in the -P and NF-soils than in TSP-fertilized soils, even lower in the latter than the T0 value (Fig. 4a).





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three independent experiments (One-way ANOVA with Tukey's test, n= 3, P < 0.05; different letters indicate significant differences between the analysed conditions). (b) Two-dimensional plots of nMDS analyses of quantitative matrices from DGGE gel patterns for bacterial 16S rDNA, fungal 18S rDNA and archaeal 16S rDNA DGGE profiles of the rhizosphere soil, prior to the transplant (T0) and after 28 days of incubation (stress values: bacteria= 0.116; fungi=

0.081; archaea=0,06969). Treatments: -P: P-deficient plants; NF: NF-treated plants; TSP: TSP-treated plants. Blue circles: T0; black circles: -P; black squares: NP-treatment; black triangles: TSP treatment.

This behaviour differed from that of alkaline phosphatase activity, which presented significantly higher values in TSP-treated soil than -P and NF-soils, the latter displaying a value only marginally higher than that of the T0 soil (Fig. 4a). Arylsulfatase activity displayed the highest values in -P soil while the enzyme activity was similar between NF and TSP treatments, although all were higher than the T0 value (Fig. 4a). The β-glucosidase activity was significantly higher in NF- and TSP-fertilized soils than in -P and T0 soils, while the protease activity was higher in -P and NF-fertilized soils than in TSP- fertilized and T0 soils (Fig. 4a).

Regarding the microbial community structure, the nMDS analysis of DGGE gel patterns revealed that the microbial communities of T0 soil clustered separately and significantly differently ($P < 0.01$) from those analysed at the end of the plant growth period for all the studied microbial groups (Fig. 4b, Table III).

TABLE III

One-way ANOSIM global test based on Bray-Curtis similarity index of bacterial 16S rDNA, fungal 18S rDNA and archaeal 16S rDNA DGGE profiles. The values presented are the R-value (R) and the P statistic (P) of significance. R= 0 means that samples are not different, R= 1 means that sample are different and R= 0.75 means samples are different but overlapping. -P: P-deficient plants; NF: NF-treated plants; TSP: TSP-treated plants

Conditions	DGGE gel	One-way ANOSIM	
T0; -P; NF; TSP		*R*	*P*
	bacterial 16 S	0.9317	< 0.01
	fungal 18S	0.8553	< 0.01
	archaeal 16 S	0.7653	< 0.01

Archea and Bacteria microbial communities of the NF- and TSP-fertilized soils displayed greater similarity while, for fungi, the greatest similarity was observed in TSP-fertilized soil and -P soils (Fig. 4b).

DISCUSSION

Low P availability (3.0 mg kg^{-1}, Fig. 3) in the soil used for cucumber plant growth led to the lowest plant shoot growth and P content in roots and shoots at the end of the experiment compared to NF- and TSP-fertilized soils (Fig. 1, 2; Table II). Both the novel NF and conventional TSP treatments were equally effective in preventing P-deficiency symptoms, as confirmed by their similar SPAD index values (Fig. 2b). Indeed, one of the symptoms of P deficiency is the intensification of the leaf colour due to the concentration of chlorophyll and excessive accumulation of anthocyanins (Hecht-Buchholz, 1967).

Similar effectiveness was observed of the NF and TSP in terms of morpho-physiological responses to P nutrition for cucumber plants, although the NF released less Olsen P, generally considered as the plant available P fraction (Wolf and Baker, 1985). This result, whether on the TSP or the NF P source, confirmed previous results obtained in hydroponic experiments showing that the P contained in the NF was used by cucumber plants because of their ability to dissolve the NF, probably due to the presence of low molecular weight organic acids at the apoplast level, and to acquire the released P (Sega *et al.*, 2020). Dissolution of NFs by low molecular weight organic acids typically present in the root exudates have been reported in previous model studies (Wang *et al.*, 2016). NF and TSP could form different P fractions on fertilization since they have different amounts of phytoavailable P, as estimated by the Olsen method and previously reported by Ciurli *et al.* (2022). The sufficiency of the P uptake in the cucumber plants, fertilized with NF or TSP, may be explained by the release of phytoavailable P by both NF and TSP, although in different chemical forms.

In fact, bulk TSP granules dissolve in a linear manner and release phosphate anions after application, following which new P-containing solid phases are formed by the reaction of phosphate with soil minerals (Degryse *et al.*, 2013). Dissolution is a main difference between TSP and NF since the latter does not readily dissolve into the soil solution and should not be sorbed in the soil solid phases (Johnston and Richards, 2003). Recena *et al.* (2015) showed that P uptake by cucumber plants was positively correlated with its affinity for the soil solid phases, particularly with Fe oxides. Conversely, the higher initial P availability caused by TSP mainly depends on the soil P buffer capacity: the release of P in the soil solution triggered by the induced

modification of the chemical equilibrium of the plant root between phosphates and the soil solid phases (Ehlert *et al.*, 2003). Globally, in terms of P fractionation in soil, the main difference between TSP and NF is this: with TSP, it is influenced by its dissolution rate and any salt precipitation due to the soil's properties while, with NF, the P fractions depend more on the physico-chemical properties of the nanoparticles that could be solubilized by the root activity, depending on the plant's nutritional demand (Sega *et al.*, 2020; Khan *et al.*, 2022). This hypothesis appeared to be supported by the results reported by Montalvo *et al.* (2015), who found, in two strongly P sorbing soils, a higher P uptake in roots of wheat plants fertilized with TSP compared to plants fertilized with hydroxyapatite nanoparticles, a less soluble P form. Rapid and high rates of P solubilization from TSP could also explain the significantly higher P accumulation in roots and shoots of plants fertilized with TSP rather than NF (Table II), since the acidic pH value of the soil used should maximize the TSP dissolution (Degryse and McLaughlin, 2014). It is known that plant roots also acquire Fe-complexed P by the release low molecular weight organic acids (Chiou and Lin, 2011) and phenolic compounds (Tomasi *et al.*, 2008).

Significantly higher concentrations of Fe and Mn in the roots of plants grown on -P soil than in those grown on NF- and TSP-fertilized soils (Table II) were the typical outcome of the nutrient limiting condition (Rogers *et al.*, 2000). Since limiting P and Fe availability can induce ion imbalance in plants (Chao *et al.*, 2011) a similar elemental composition of NF- and TSP-fertilized plants could be considered a complementary indication of an optimal P supply to plants by both NF and TSP, while a lower Mg in the roots of plants fertilized with TSP could be attributed to the formation of sparingly soluble complex Mg-phosphates, though Mg cations are more active at alkaline pH values (Epstein and Bloom, 2005). It is known that P availability influences the uptake and homeostasis of metallic micronutrients such as K, Ca, Mg, Fe, Mn, Cu, and Zn (Pérez-Novo *et al.*, 2011), and that excessive P fertilization can cause micronutrient deficiency (Cakmak and Marschner, 1987). Moreover, relatively high concentrations of Mn in P deficient soils have been ascribed to the larger formation of cluster roots (Shane and Lambers, 2005). Conversely, higher Cu concentration in plants grown under P deficient conditions could be explained by the activation of low-phosphate signalling pathways, which involve the activation of Copper Transport Proteins Genes (COPT) delivering Cu to Cu-proteins that respond to low P signals (Perea-García *et al.*, 2013) while, in plants fertilized with an adequate P level, the Cu concentration is generally at normal levels (Zhang *et al.*, 2020). Globally, since significant differences in Cu and Mn concentrations were only observed between unfertilized and P fertilized and not between NF- and TSP-treated plants, the analysis of metallic micronutrients confirmed that the novel NF was able to adequately supply P as TSP to the cucumber plants.

Higher acid soil phosphatase activity in -P compared to the unfertilized and unplanted soil (T0), NF- and TPS-treated soils (Fig. 4a), could be ascribed to the secretion of acid phosphatases by soil microorganisms in order to release P from organic esters in response to its low availability (Tarafdar and Claassen, 1988; Vance *et al.*, 2003). The increase in the other measured enzyme activities in all planted soils compared to the T0 soil was related to the larger and more active microbial community in the rhizosphere, sustained by the release of root exudates and the turnover of fine roots. In pot experiments, the soil can be considered to be fully colonized and influenced by the presence of roots (Daly *et al.*, 2015). The alkaline phosphatase activity is mainly produced by soil microorganisms (Tarafdar and Claassen, 1988), and this activity increases phytoavailable P (Margalef *et al.*, 2017). The increase of protease activity in the planted soils could be related to the microbial metabolic activity (Trasar-Cepeda *et al.*, 2008). An increase of protease activity in soils amended with nano-TiO_2 and nano-ZnO was reported by Ge et al. (2013), but a range of effects on soil protease activity of different nanoparticles has been reported in the rhizosphere of different plants (Asadishad *et al.*, 2018).

Changes in the archaeal, bacterial and fungal communities of the planted soils compared to those of the T0 soil (Fig. 4b) confirmed the colonization of the soil by the cucumber roots, even in -P soil. The selection of microbial communities in the rhizosphere is a well-known phenomenon driven by the proliferation of selected microbial groups, capable of using root exudates and rhizodeposition as growth substrates (Berg and Smalla, 2009).

Differences between the microbial communities of planted soils under different treatments at the end of the plant growth period could be ascribed to changes in the root exudation profile of plants supplemented with nutrients from different fertilizer types. For example, McKnight *et al.* (2020) reported that nanoapatite used as P nanofertilizer exerts greater influence on the microbial community of the soybean plants rhizosphere than that of a plant fertilized with the bulk form of apatite fertilizers.

CONCLUSIONS

The novel tested P NF proved to be as effective as TSP in sustaining the growth of cucumber plants since no significant morpho-physiological differences were observed between plants supplied with the two fertilizers during plant development (Fig. 5).

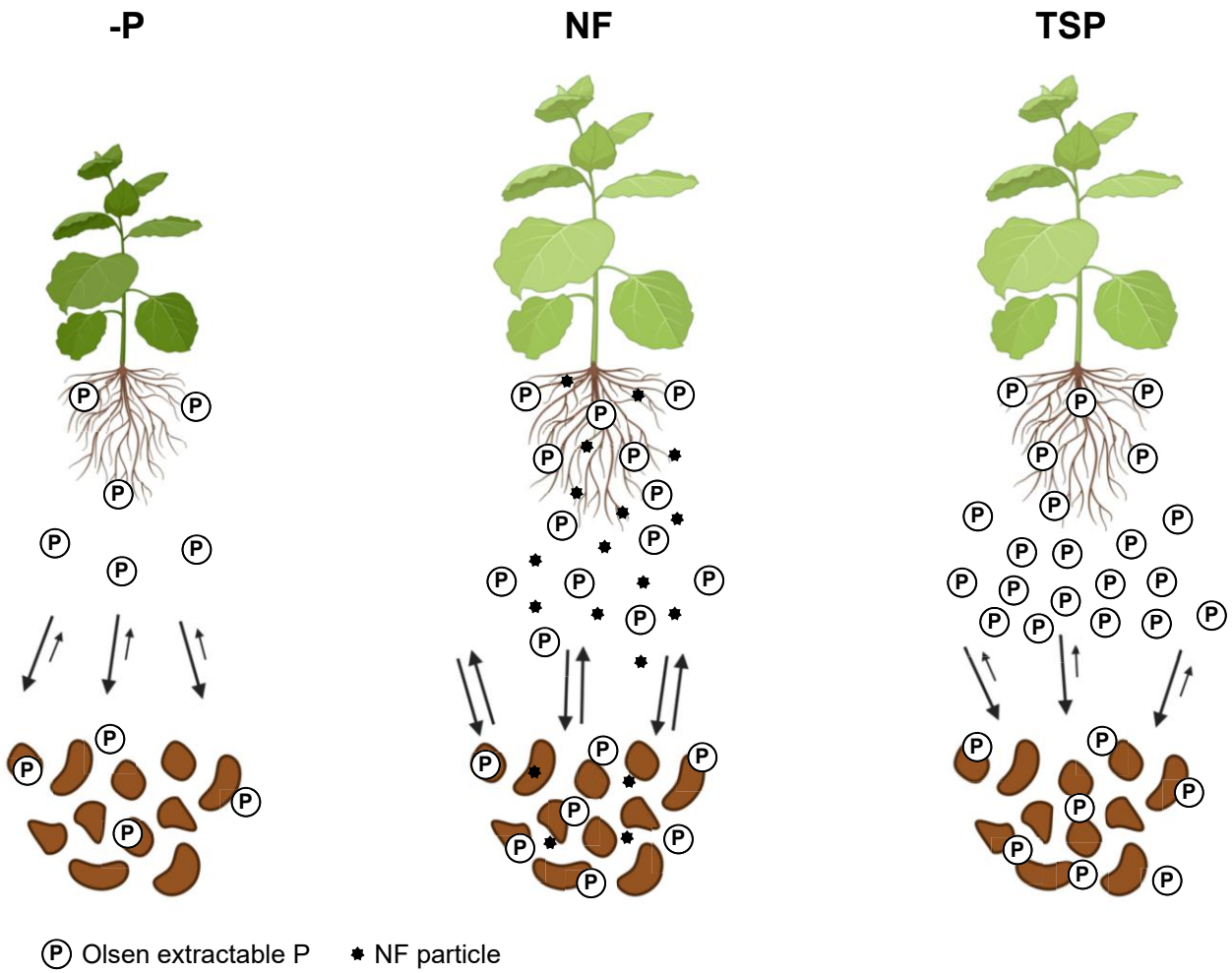

Fig. 5 Conceptual model explaining the NF and TSP interaction in the plant-soil system. The diagram depicts the possible mechanisms involved in P nutrition. Specifically, TSP releases a higher quantity of P Olsen in available form that can, in turn, be acquired or subjected to absorption/precipitation in the soil. The NF can directly interact with cucumber root apoplast/exudates and release P more slowly, either in solution or after absorption.

The NF releases P in soil more slowly than and in different chemical forms to the TSP fertilizer, probably less considered in the Olsen method but equally phytoavailable. This finding confirms the need for an improvement in the methods of assessing phytoavailable P from NFs in the future. Parallel lower P and Fe concentrations in the roots of plants fertilized with NF indicate that the dissolution dynamics of $FePO_4$ nanoparticles could be under the control of the root activity, and future research is needed to understand whether NF induces changes in the exudation profile of plant roots. If confirmed, such differences could explain the variation in the microbial community structure of the rhizosphere of the NF- and TSP-fertilized plants observed in this study. Overall, the results suggest that the $FePO_4$-NPs-based NF tested can be an effective alternative to conventional TSP fertilizers.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

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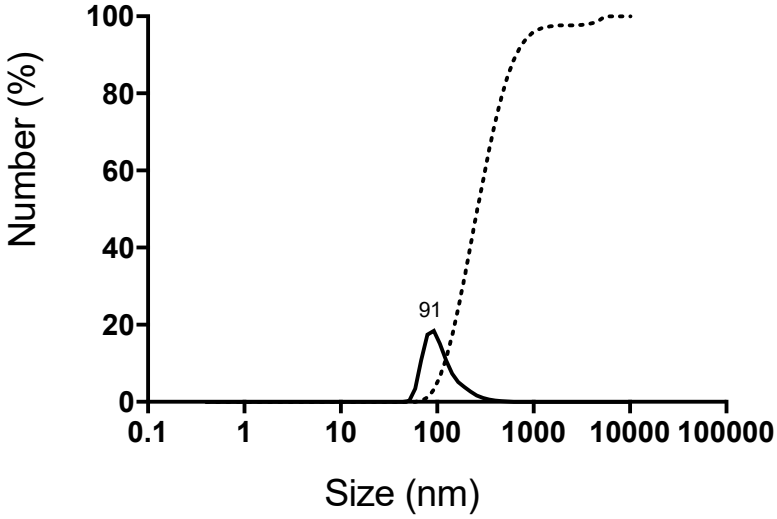

Fig. S1 Size distribution of NPs determined through DLS analysis.