UNIVERSITÀ di VERONA
Dipartimento di BIOTECNOLOGIE

FABBRICA COOPERATIVA PERFOSFATI CEREA

$FePO_4$ nanoparticles as an effective tool for plant nutrition: from the lab to the field

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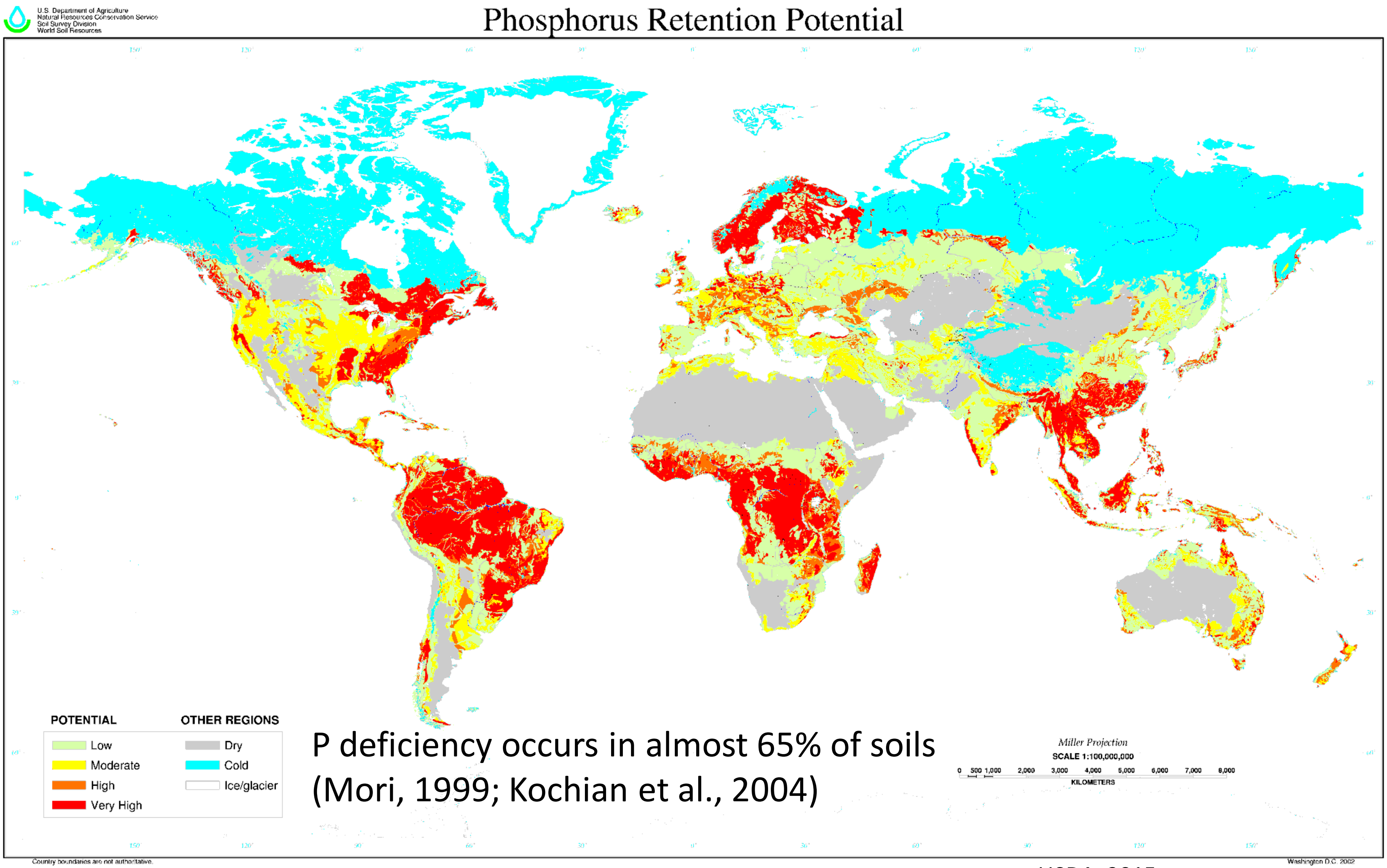
50 nm

P and Fe: essential mineral nutrients

Often scarcely available for plants

Behavior of P in soil

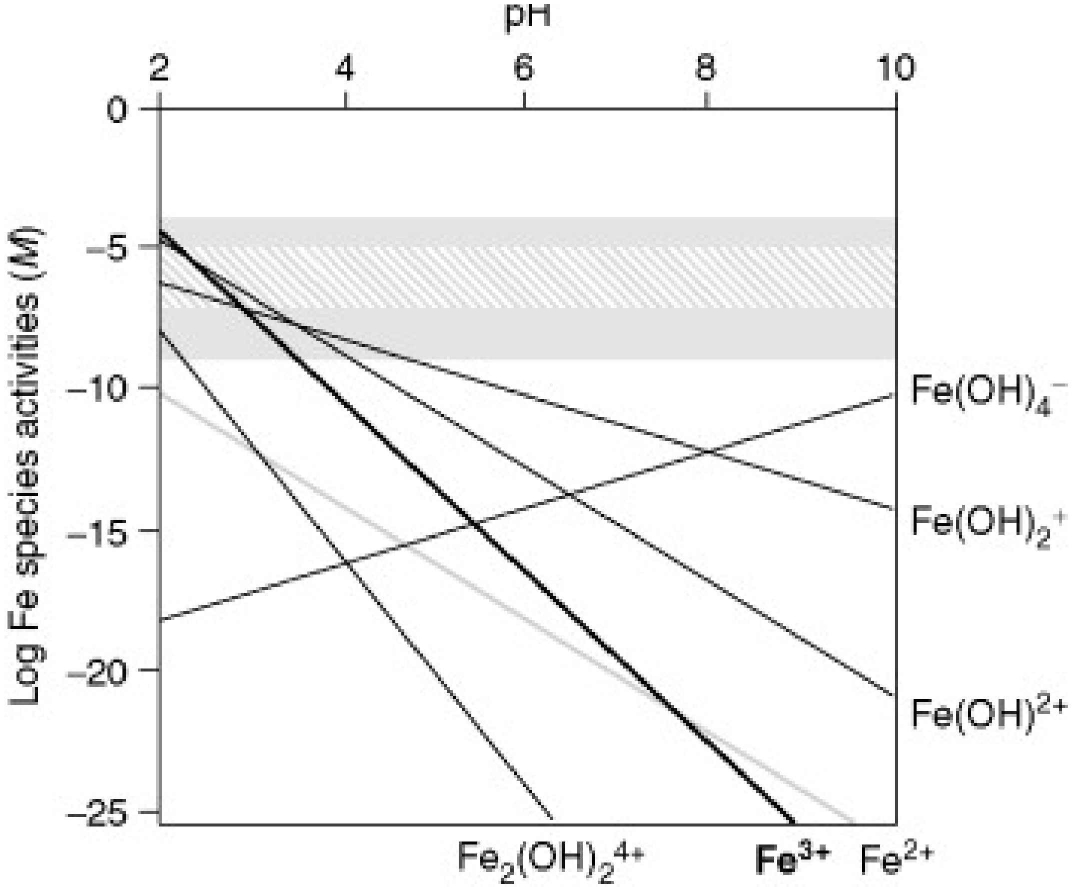


P deficiency occurs in almost 65% of soils (Mori, 1999; Kochian et al., 2004)

USDA, 2015

Behavior of Fe in soil

$$Fe(OH)_3 \leftrightarrow Fe^{3+} + 3OH^-$$



Fe deficiency occurs in about 30% of soils (Mori, 1999)

Limits of current P and Fe fertilizers

P

-Sequestration in acidic and calcareous soils

-Phosphate rock is not renewable!

Fe

-$FeSO_4$: rapid oxidation of Fe^{2+} to Fe^{3+} with consequent precipitation

-Chelates: high price and risk of leaching

Why nanotechnology?

Research Article

Received: 21 July 2011 Revised: 6 October 2011 Accep

(wileyonlinelibrary.com) DOI 10.1002/jsfa.5569

Pot evaluation of synt
prevention of iron chl

**Inmaculada Sánchez-Alcalá,* María
and José Torrent**

SCI

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:

Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean

, the Ohio State University, 210 Kottman

: phosphorus (P) fertilizers, cause
supplying the nutrient P. In
ient P nutrients to crops but with
comparison to the soluble

frontiers in Plant Science

ORIGINAL RESEARCH
published: 09 June 2016
doi: 10.3389/fpls.2016.00815

CrossMark

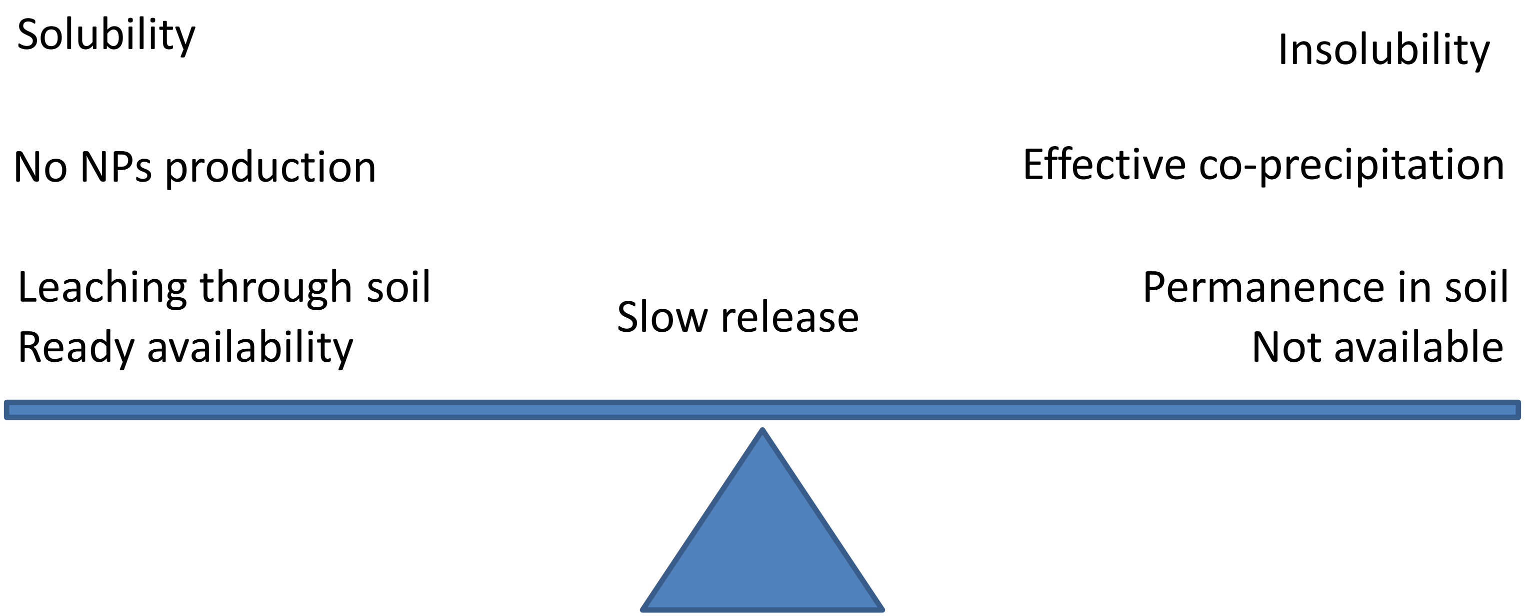
Iron Oxide Nanoparticles as a Potential Iron Fertilizer for Peanut (*Arachis hypogaea*)

Mengmeng Rui[1,2], Chuanxin Ma[3], Yi Hao[1], Jing Guo[4], Yukui Rui[1,3], Xinlian Tang[2], Qi Zhao[1], Xing Fan[1], Zetian Zhang[1], Tianqi Hou[1] and Siyuan Zhu[1]*

What are the characteristics of the ideal fertilizer?

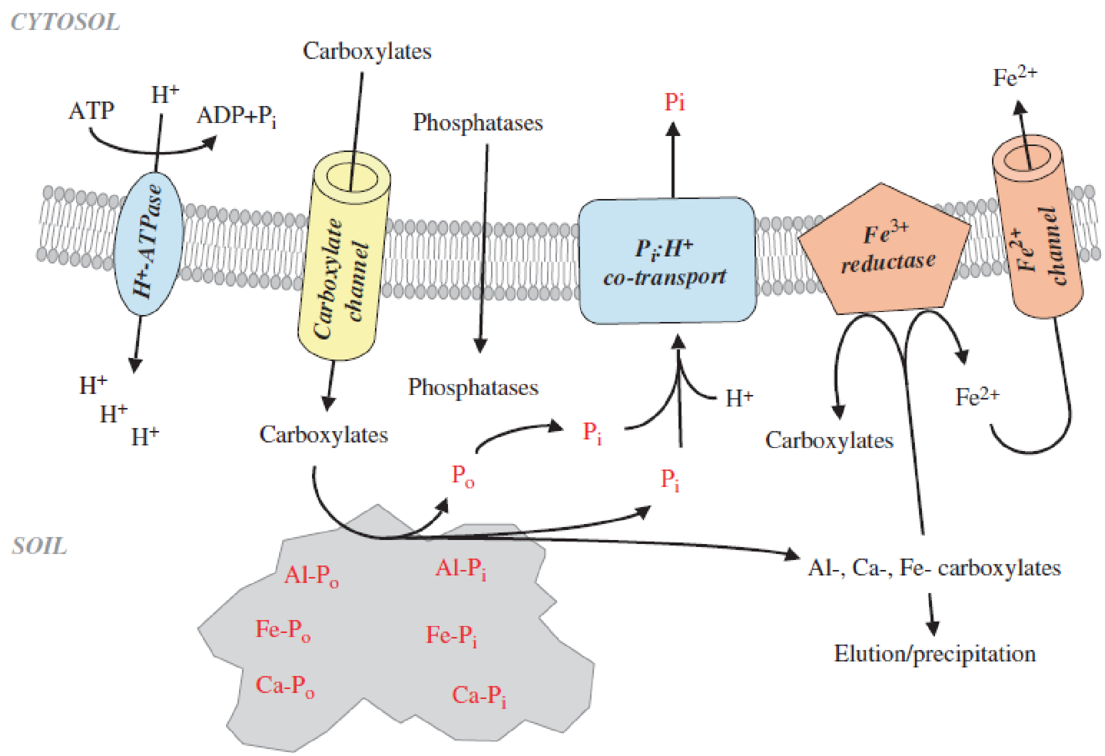
- Easy production
- Easy application
- Low cost
- Good availability for plants
- Effectiveness over time (slow release of nutrients)
- Absence of leaching
- Absence of toxicity (plants, microbes, other organisms)

Delivered when needed



Why $FePO_4$?

- Low solubility ($K_{sp} = 1.3 \cdot 10^{-22}$)
- It is solid -> It has a low reactivity in soil solution
- Plants can mobilize P and Fe from $FePO_4$ when readily available sources are not enough

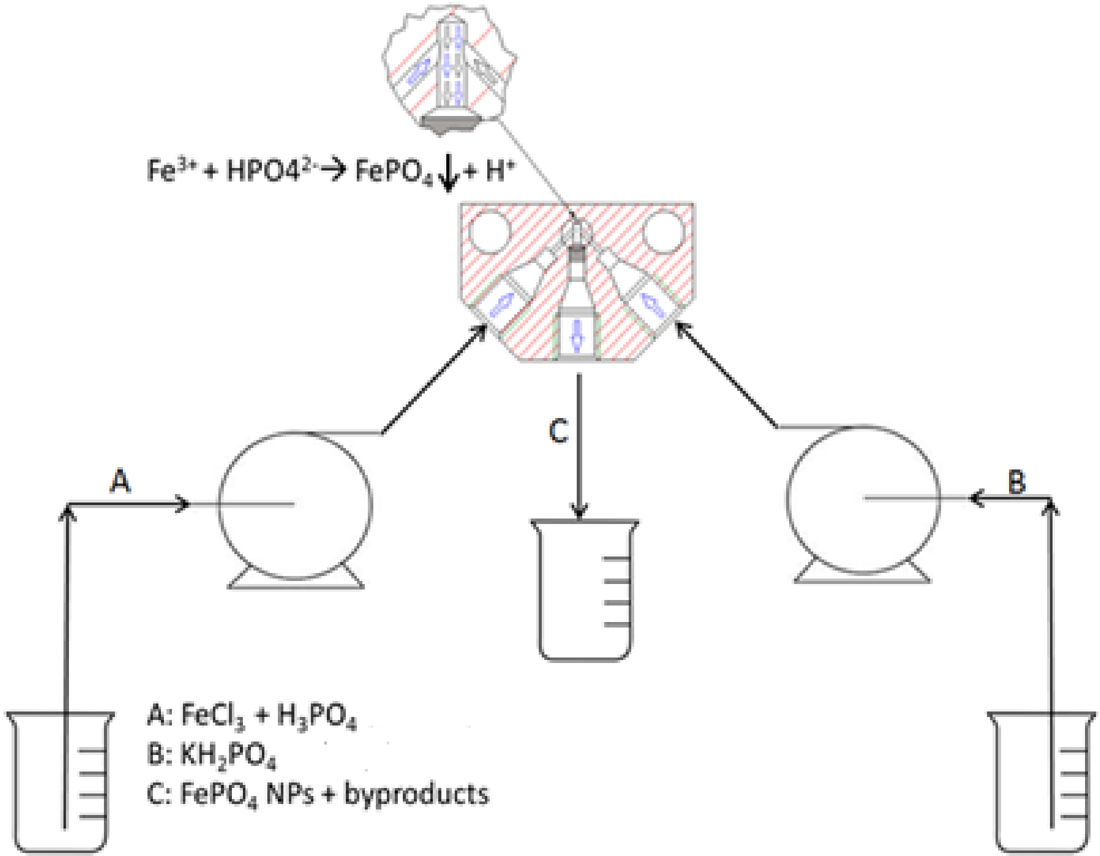


Joint Project 2014 UniVR-FCP Cerea "Nanofert"

Study the capacity of $FePO_4$ NPs to deliver P and Fe to plants, for understanding their potential as innovative fertilizer.

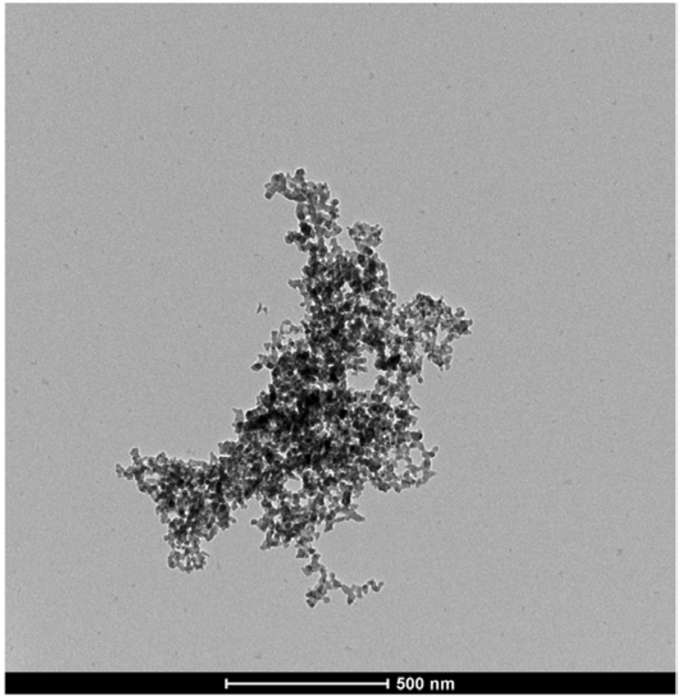
Optimize a simple, economically advantageous and industrially scalable synthesis method for producing $FePO_4$ NPs, that could provide a product with a convenient shelf-life making it potentially exploitable in the fertilizer market.

Continuous-flow synthesis with a pilot plant

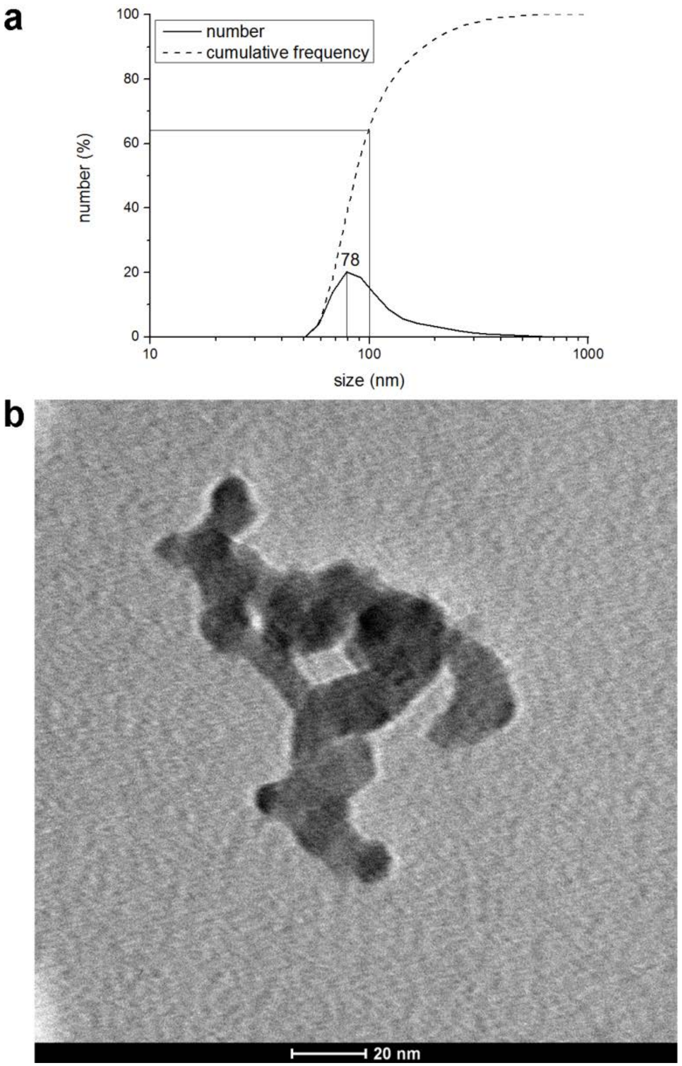


Productivity of 15 $L \cdot h^{-1}$ of suspension, accounting for approx 140 g $FePO_4$ $NPs \cdot h^{-1}$

Continuous synthesis with a pilot plant



Purification through centrifugation



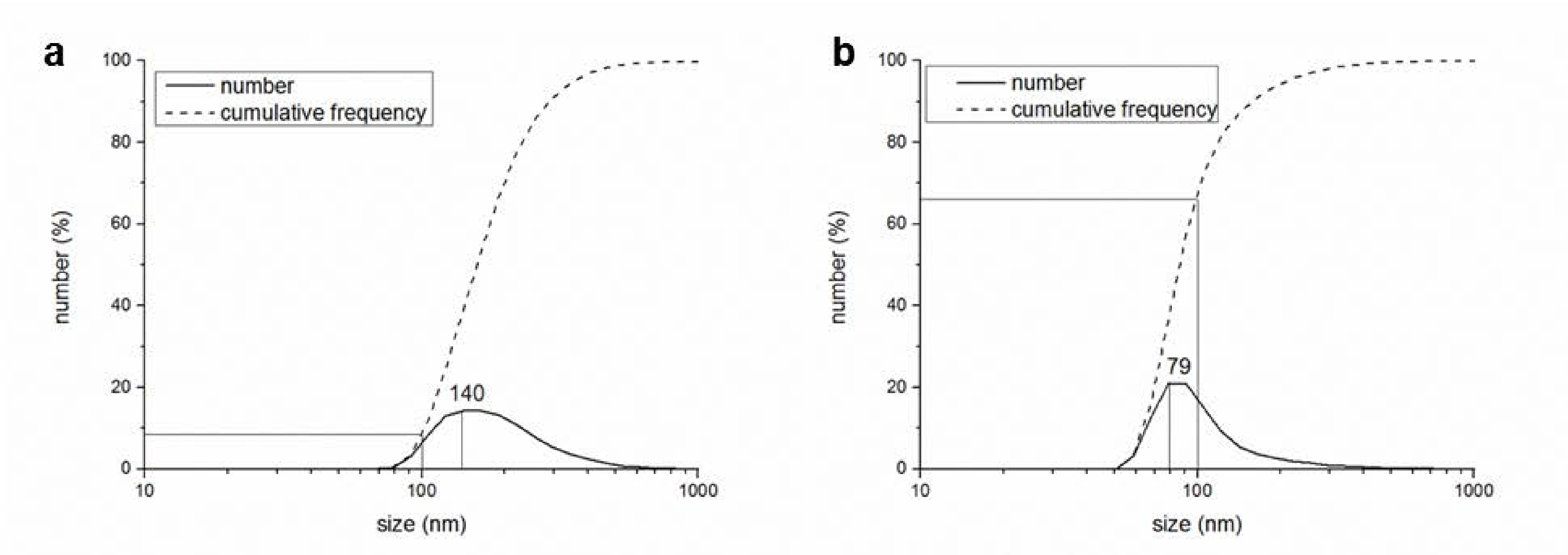
Purification through dialysis

Sega et al., 2019

Stability of NPs on long time period

Bare NPs

Citrate-capped NPs

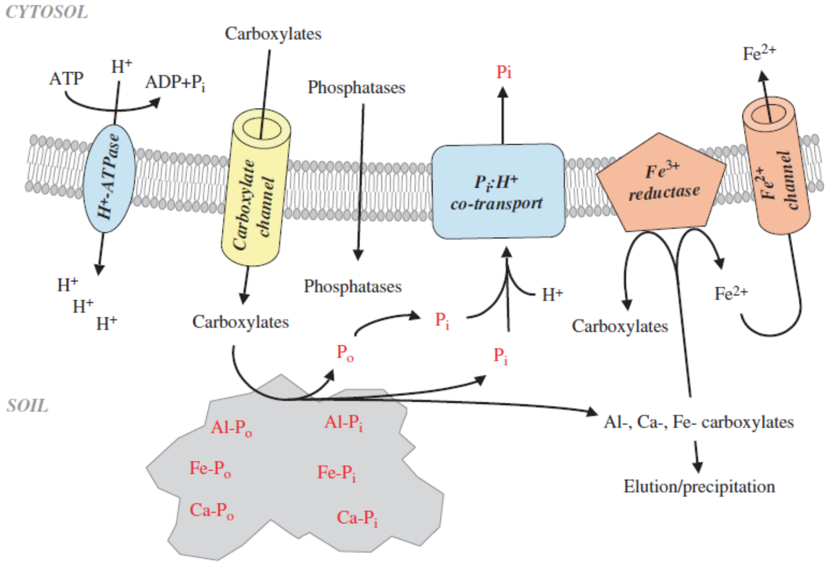


Size distribution of NPs after 8 months of storage

Sega et al., 2019

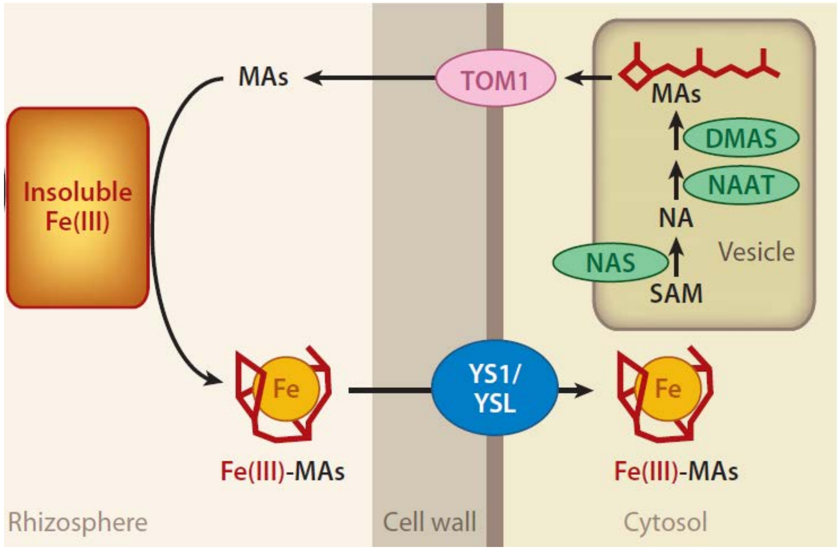
Effects on plants: choice of the species

Strategy I: Dicotyledons and non-graminaceous monocotyledons



Cucumber

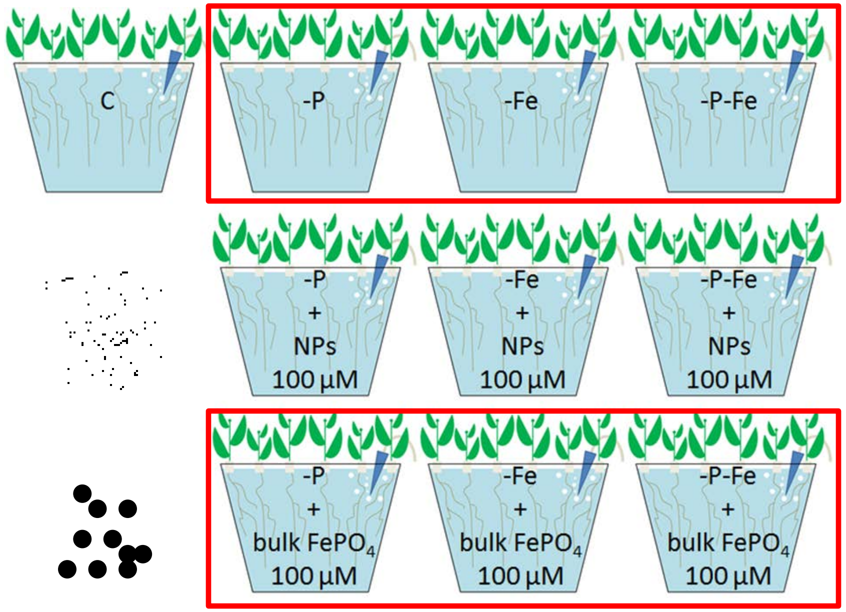
Strategy II: graminaceous monocotyledons



Maize

Choiche of the experimental setup

Germination (6 days)	Treatment (14 days)	Sampling
24 °C, $CaSO_4$ 1mM, dark	25 °C, 16h light/8h dark	



Control plants were grown with 100 µM of PO_4^{3-} and 100 µM FeNaEDTA.

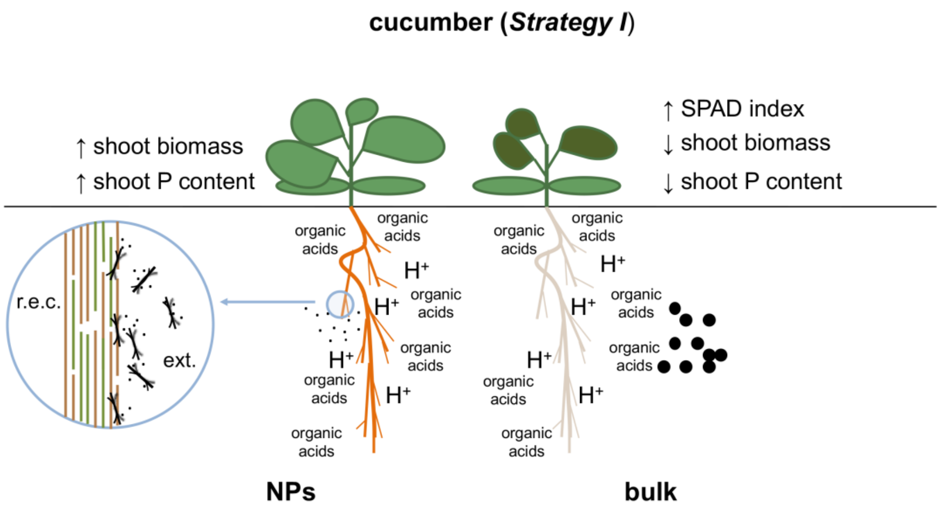
Three indipendent growth experiments were set up

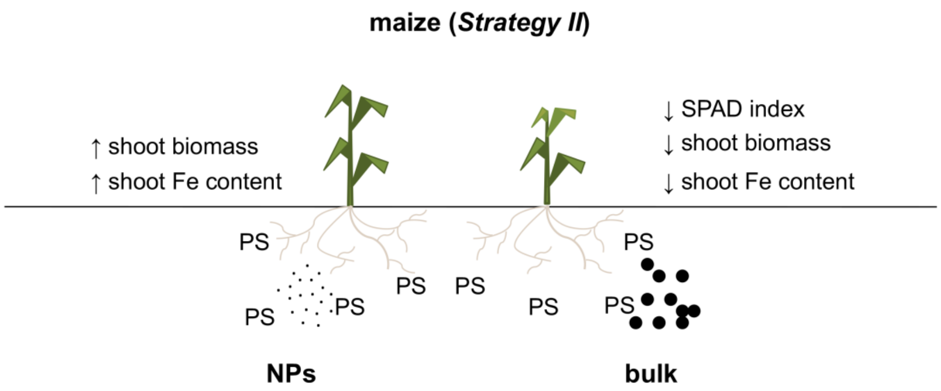
-Physio-morphological parameters

-Multielement analysis of plant tissues through ICP-MS

Sega et al., 2020 *accepted*

Effects of $FePO_4$ NPs on cucumber and maize



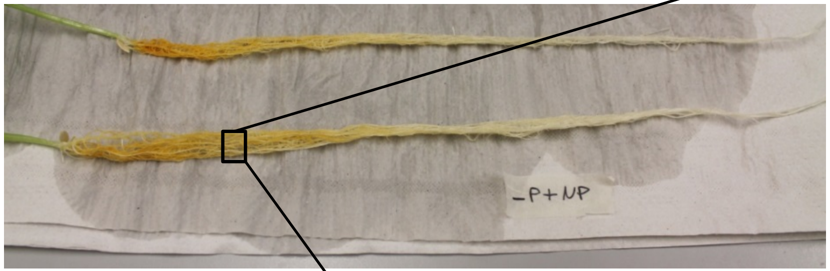


	P			
	+	-	NPs	bulk
Morphology	xxx	x	xxx	x
Nutrient content	xxx	x	xx	x

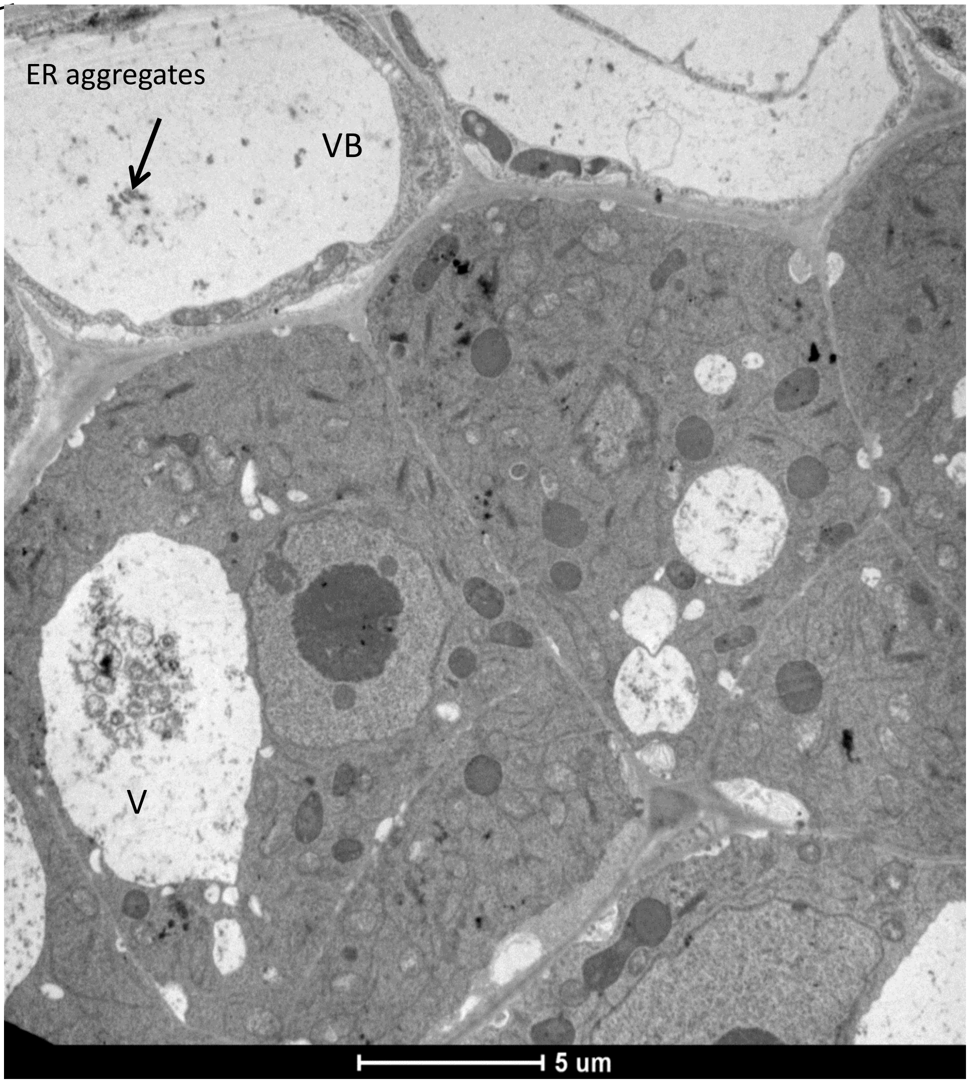
	Fe			
	+	-	NPs	bulk
Morphology	xxxx	x	xxx	xx
Nutrient content	xxxx	x	xxx	xx

Sega et al., 2020 *accepted*

Do nanoparticles enter into the plant?



Root orange coloration of P deficient cucumber plants treated with $FePO_4$ NPs

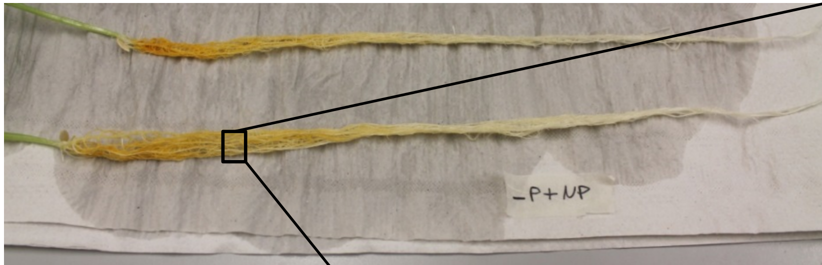


TEM image of a cross section of tertiary cucumber roots grown with $FePO_4$ NPs as P source (-P+NPs). Image: Barbara Baldan (UniPD)

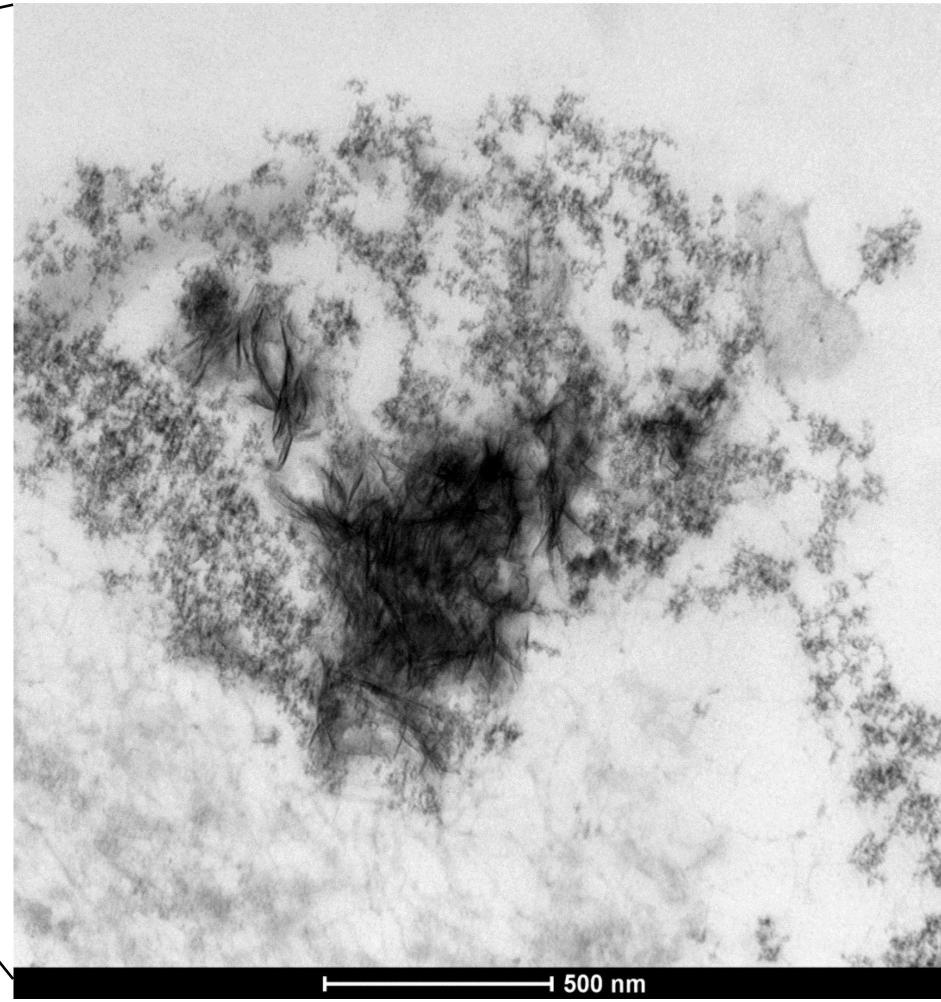
No NPs were observed into the roots of any treatment

Sega et al., 2020 *accepted*

Do nanoparticles enter into the plant?



Root orange coloration of P deficient cucumber plants treated with $FePO_4$ NPs

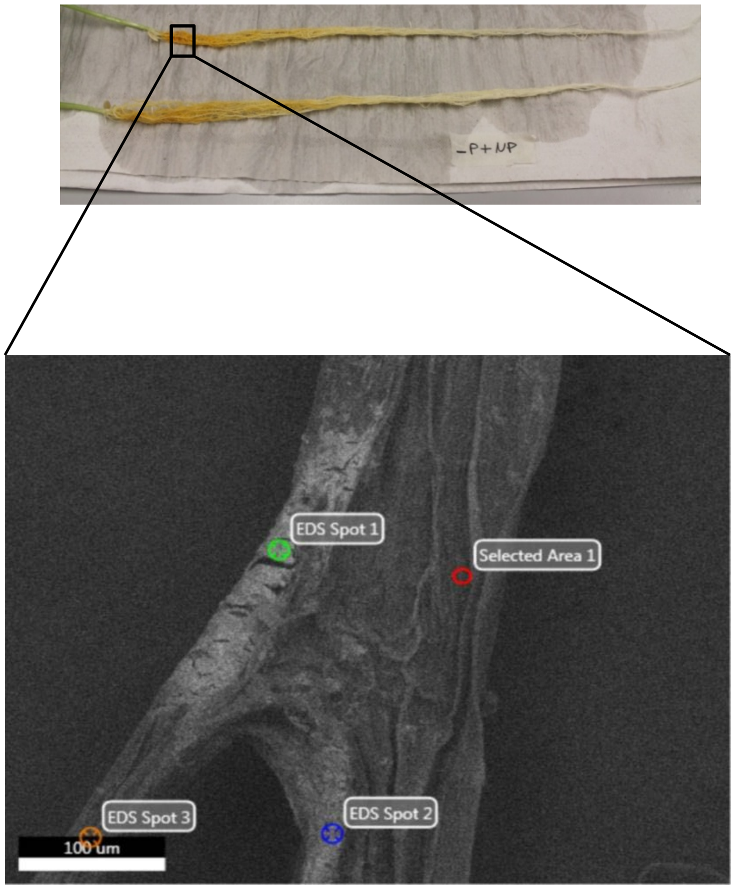


TEM image of a cross section of tertiary cucumber roots grown with $FePO_4$ NPs as P source (-P+NPs). Detail of black laths together with other nano-sized granules outside the cell wall. Image: Barbara Baldan (UniPD)

Instead of our nanoparticles, other nano-sized objects were observed

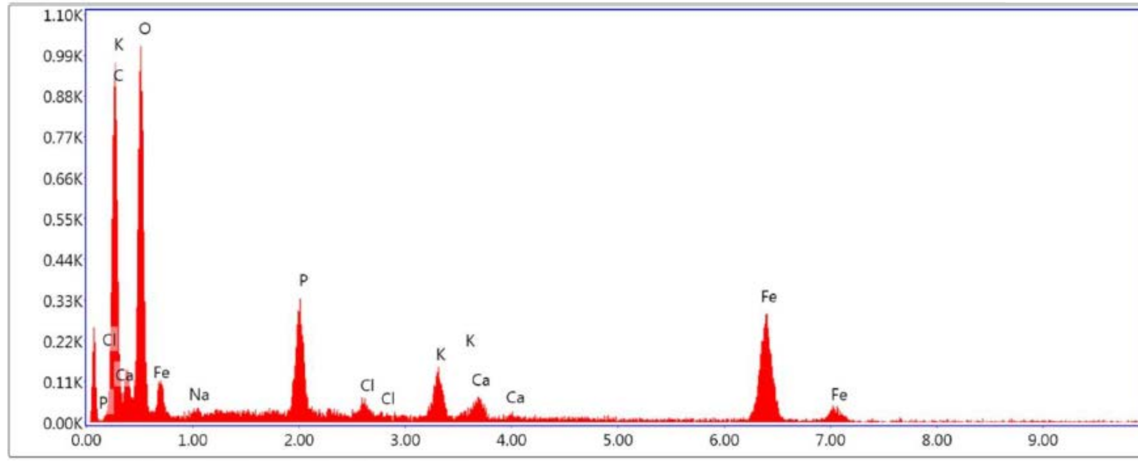
Sega et al., 2020 *accepted*

ESEM observation and EDAX analysis

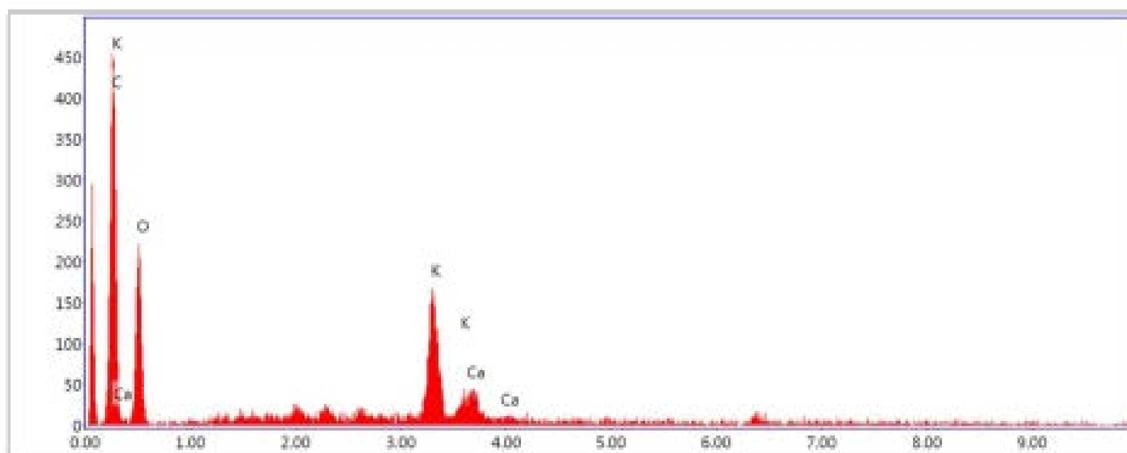


ESEM observation of roots of cucumber plants grown with $FePO_4$ NPs as P source (-P+NPs); notice the electron dense crusts.

EDS Spot 1



Selected Area 1

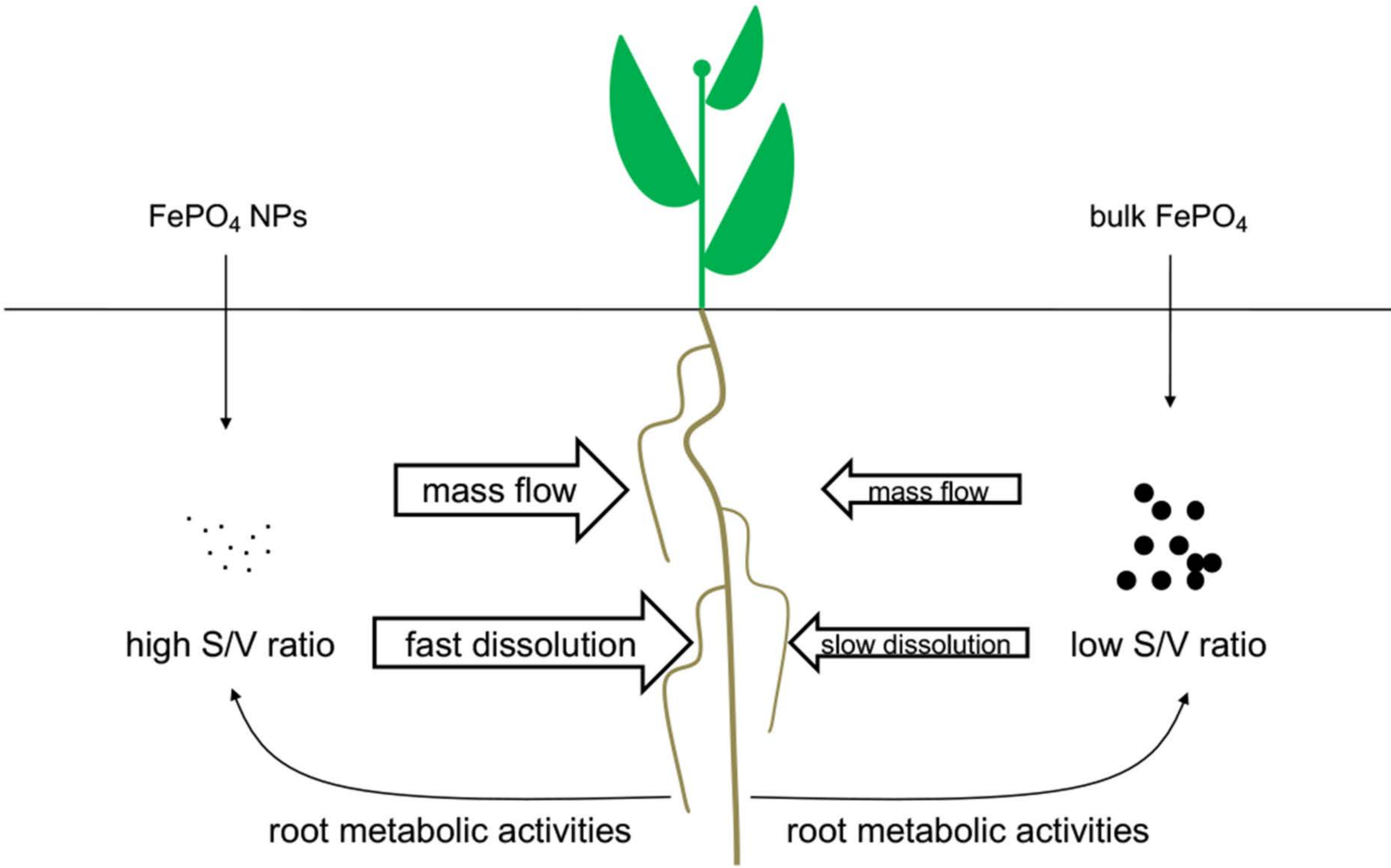


EDAX analysis of roots of cucumber plants grown with $FePO_4$ NPs as P source (-P+NPs).

Fe/P atomic ratio between 2.49 and 3.84

Sega et al., 2020 *accepted*

Nutrients delivery by $FePO_4$ NPs in the plant-soil system

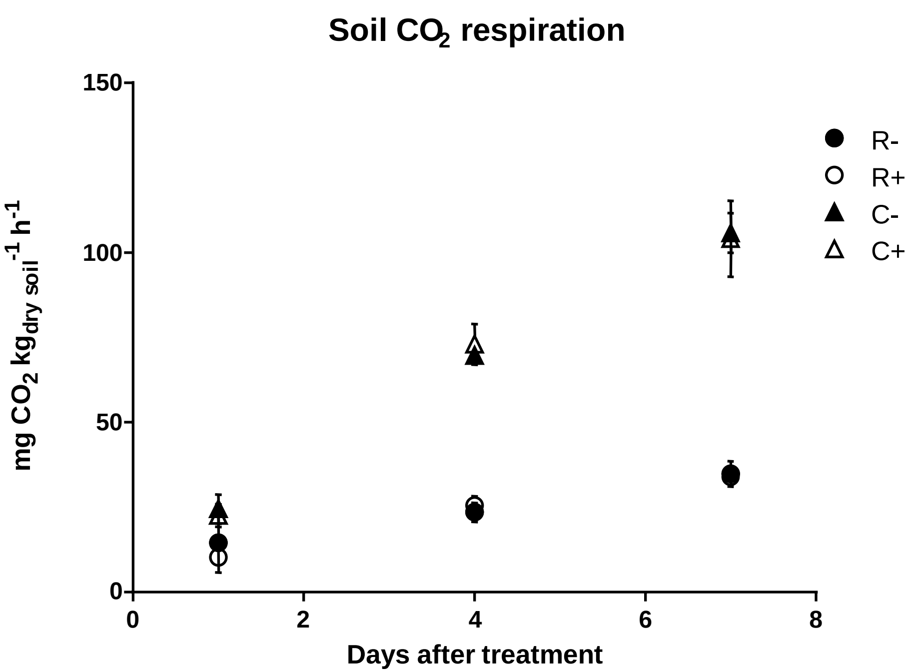


Sega et al., 2020 *accepted*

Metabolic activity of soil microbiome

Two bare soils were treated and incubated for 7 days: a sandy one (ROMOLA) and a silt loam one(CESA)

Rate: 34 mg P/kg soil



ROMOLA: (R)
CESA: (C)

Control (-) only water

NPs (+) application of $FePO_4$ NPs

- ✓ $FePO_4$ NPs did not alter soil's respiration rate
- ✓ BIOTOX^{TM} test confirmed the absence of toxicity effects

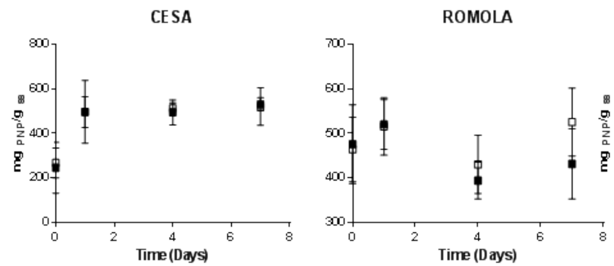
Ciurli et al., unpublished

Enzymatic activities

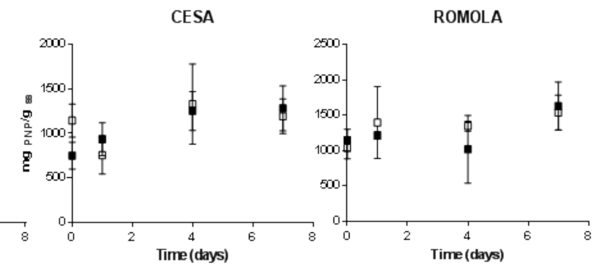
Rate: 34 mg P/kg soil

□ Control
■ Nanoparticles

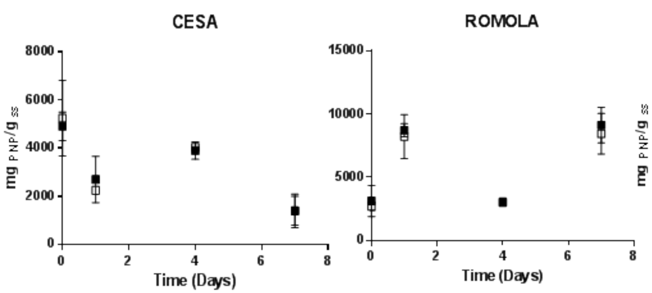
Arylsulfatase



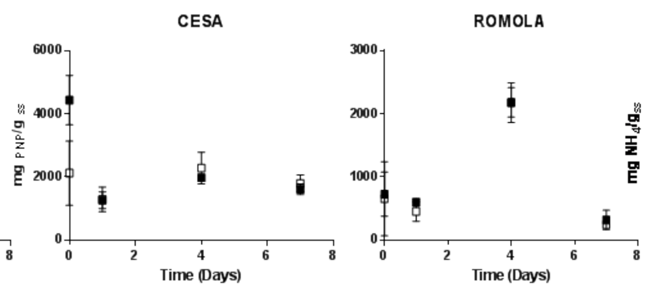
β-Glucosidase



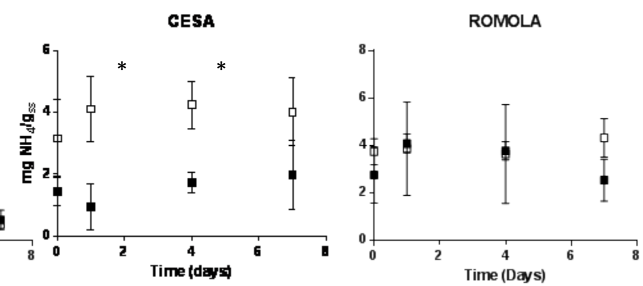
Acid phosphatase



Alkaline phosphatase



Protease



No significant variations were detected in soils treated with $FePO_4$ NPs

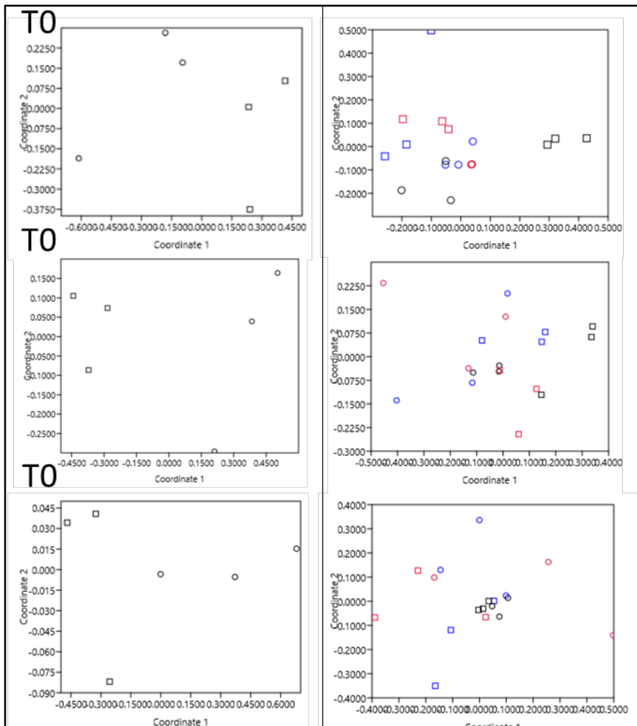
Ciurli et al., unpublished

Microbial communities

2-D plots of nMDS analyses perfoormed on DGGE patterns

Rate: 34 mg P/kg soil

CESA

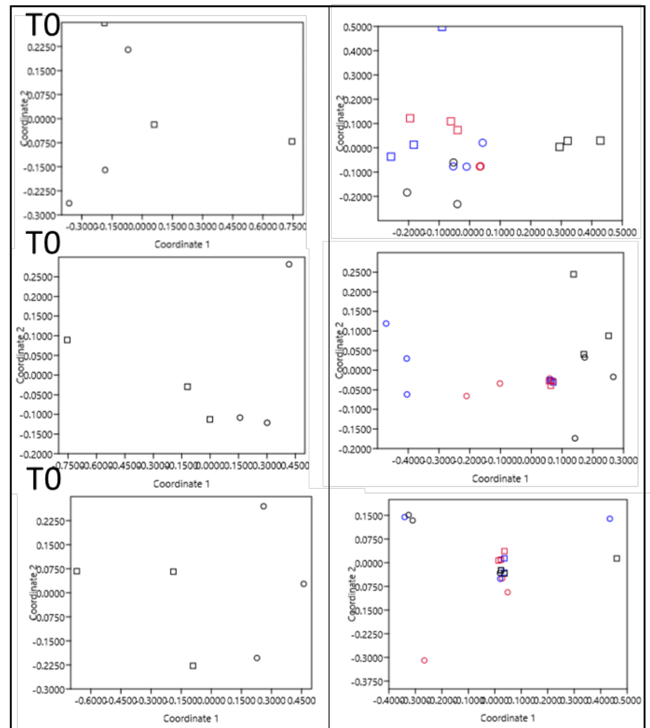


Bacteria 16S

Fungi 18S

Archaea 16S

ROMOLA

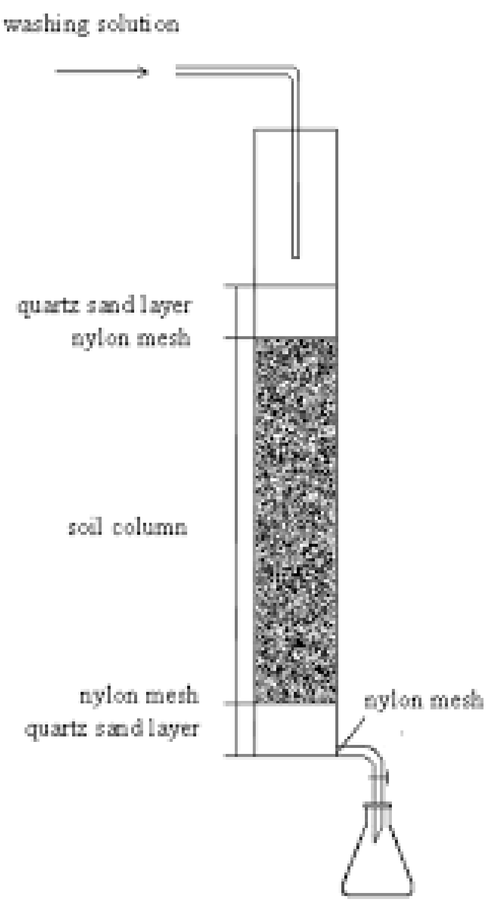


CIRCLE	Control
SQUARE	$FePO_4$ NPs treatment
BLUE	T= 1gg
RED	T= 4 gg
BLACK	T= 7 gg

No significant variations in microbial communities were detected after treatment with $FePO_4$ NPs

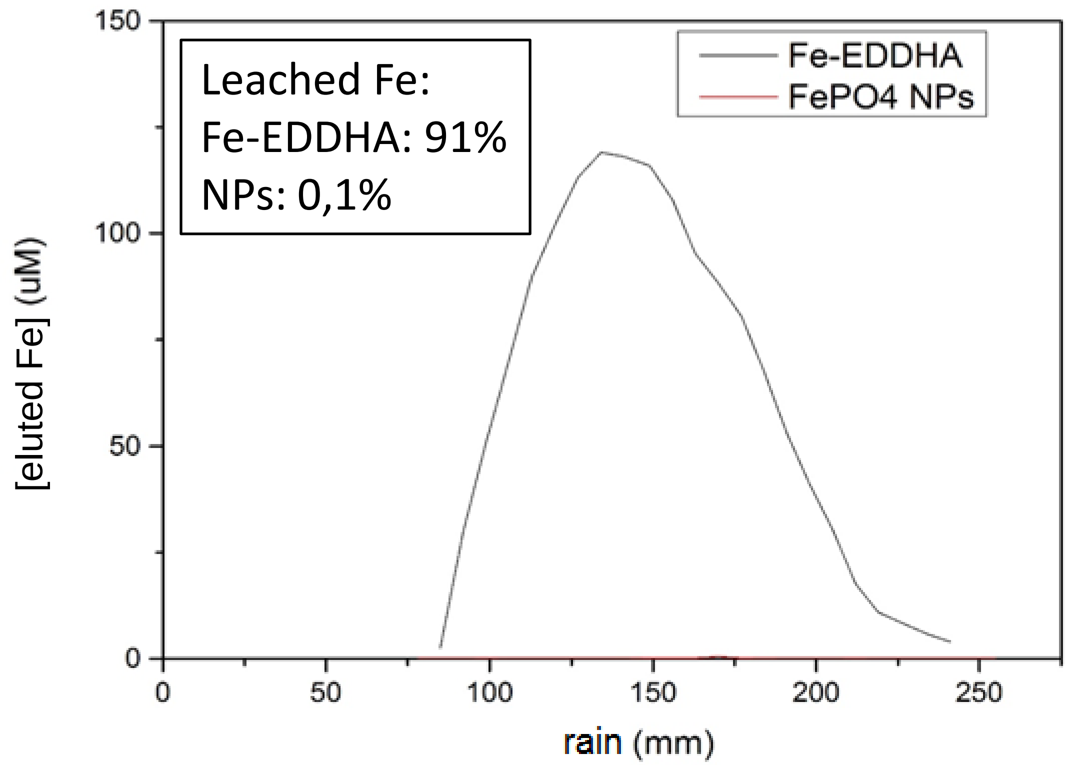
Ciurli et al., unpublished

Leaching through soil profile?



Soil column
About 400g soil
h=39cm
Ø=4cm
V≈275 cm^3

7.7umol applied Fe
About 600 mg_{Fe}/m^2
6 kg_{Fe}/ha
100 $kg_{FeEDDHA}/ha$



A summary of results

$FePO_4$ NPs:

- Can be produced in an industrially scalable way;
- Are an available source of P and Fe in hydroponics;
- Do not enter into roots – they are dissolved outside;
- Are not toxic for soil microbial communities;
- Do not leach through soil profile.

What about the field?

-Field trial on kiwifruit (jintao variety) orchard

-Sensitive variety to active limestone

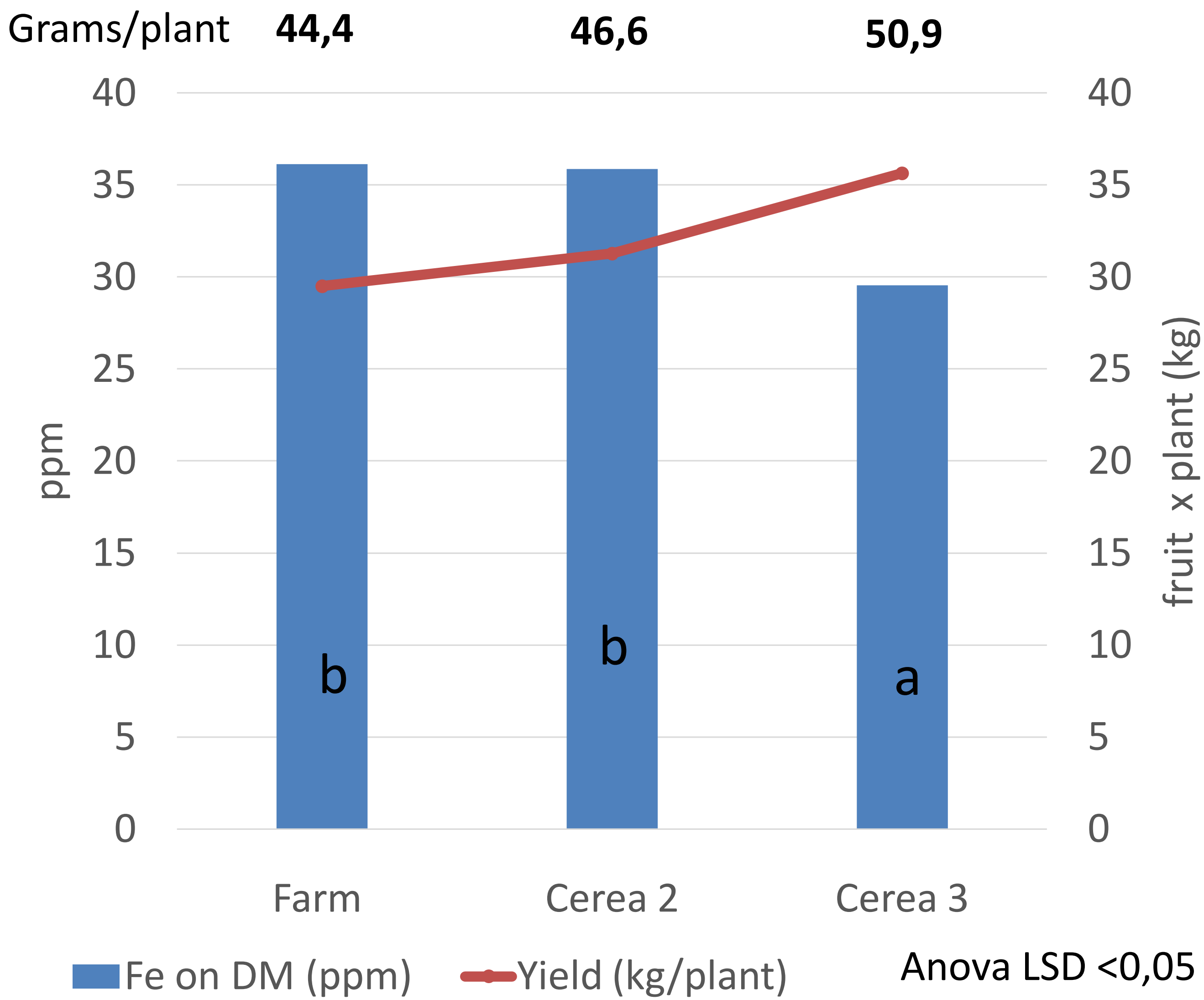
- High active limestone content in soil

										Active ingredient Kg/ha		
THESES	PRODUCT	Kg/ha	N UREIC %	$[K_2O]$ %	$[P_2O_5]$ %	[Fe] %	CHELATED %	Orto orto %	Orto para %	Fe EDDHA	NANO.T FE	Delta %
Farm	Sequestrene[1] 138 NK Fe	24	3,0	15,0		6,0	5,5	3,0	1,9	1,176		
Cerea 2	NaNo.T 1,7 [2]	30		2,8	2,1	1,7					0,504	42,9
Cerea 3	NaNo.T 1,1 [2]	45		1,0	1,4	1,1					0,504	42,9

[1]Syngenta commercial product (Fe chelate)

[2]FCP Cerea commercial product ($FePO_4$ NPs)

What about the field?



Anova LSD <0,05

Acknowledgements

-Fabbrica Cooperativa Cerea Perfosfati: framework of the joint project “Nanofert”

-Prof. Flavia Guzzo (Dept. of Biotechnology, UNIVR): precious tips for TEM analysis roots sampling

Research outcomes

-Patent N. 102018000002440 – Italian Patent Office - “Processo, e relativo impianto, per l’ottenimento di nanoparticelle di fosfati contenenti nutrienti minerali essenziali per la nutrizione delle piante”;

-Sega D., Ciuffreda G., Mariotto G., Baldan B., Zamboni A., and Varanini Z. (2019). $FePO_4$ nanoparticles produced by an industrially scalable continuous-flow method are an available form of P and Fe for cucumber and maize plants. *Sci. Rep. 9, 11252. doi:10.1038/s41598-019*-47492-y;

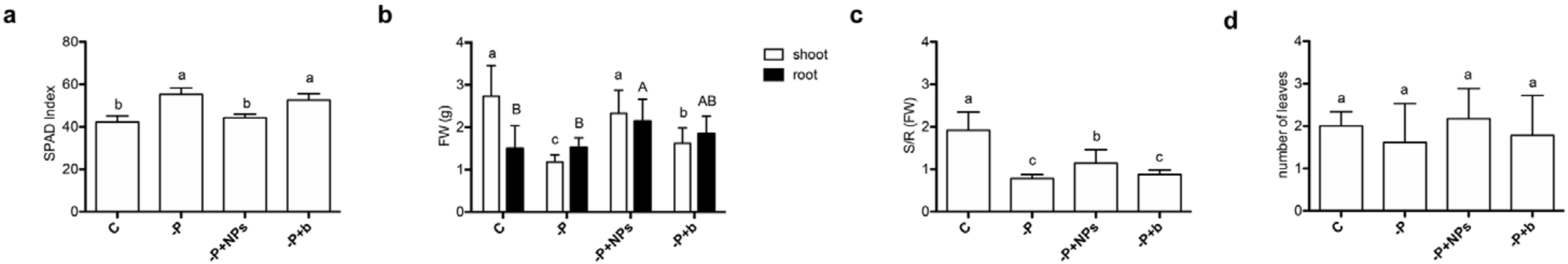
-Sega D., Baldan B., Zamboni A., and Varanini Z. (2020). $FePO_4$ NPs are an efficient nutritional source for plants: combination of nano-material properties and metabolic responses to nutritional deficiencies. *Front. Plant Sci. doi: 10.3389/fpls.2020.586470.*

Thanks for your attention!

Effects of $FePO_4$ NPs on cucumber

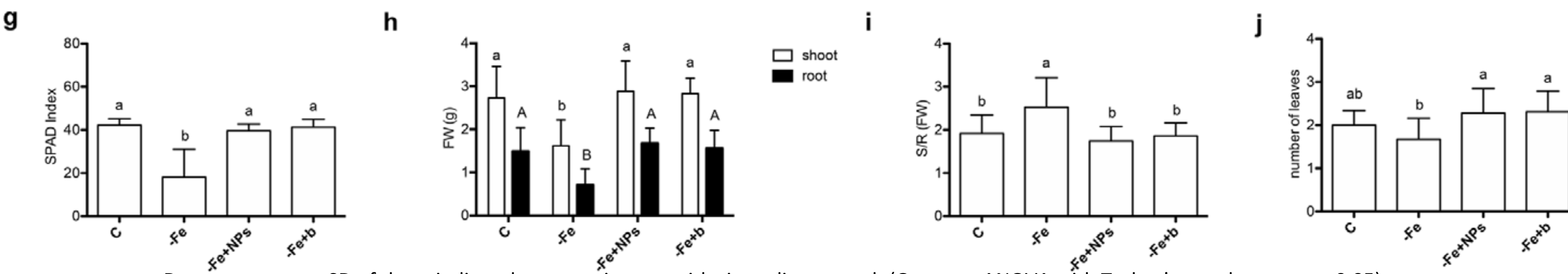
Physio-morphological effects

P nutrition



Data are means ± SD of three indipendent experiments with six replicates each (One-way ANOVA with Turkey's post hoc test, $p<0.05$)

Fe nutrition



Data are means ± SD of three indipendent experiments with six replicates each (One-way ANOVA with Turkey's post hoc test, $p<0.05$)

Plants grown with $FePO_4$-NPs as P source show less P-deficiency symptoms than plants grown with non-nano $FePO_4$

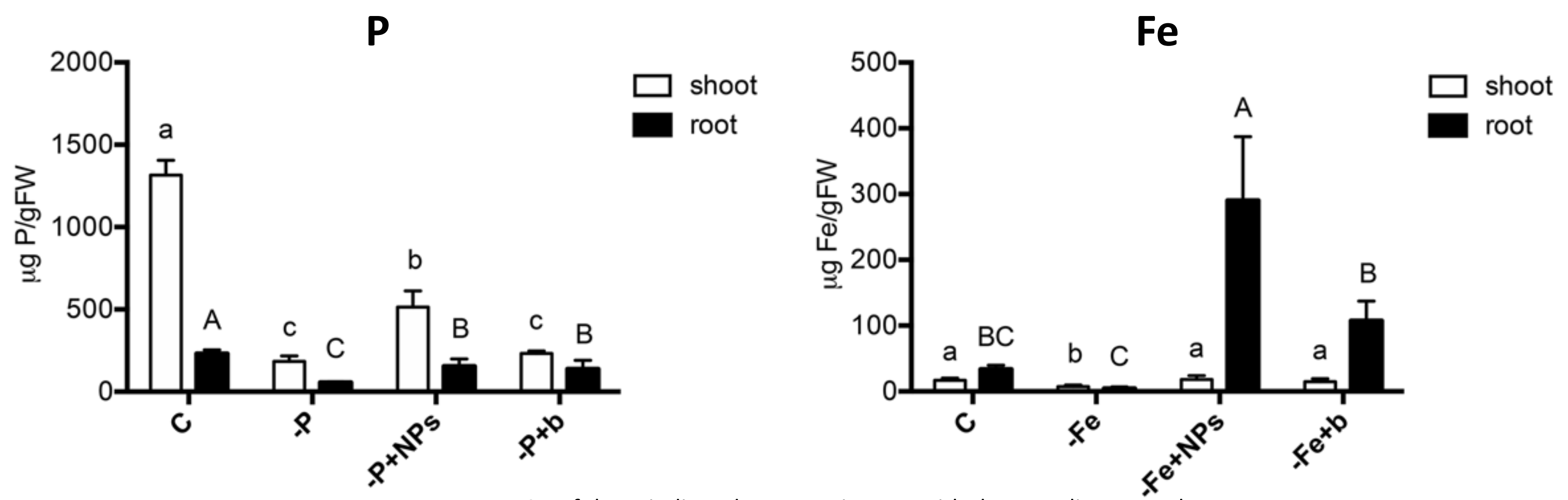
Both plants grown with $FePO_4$-NPs and non-nano $FePO_4$ as Fe source show similar values of the positive control

Sega et al., 2020 *accepted*

What about the nutrients content in plant tissues?

$FePO_4$-NPs are a more available P form than non-nano $FePO_4$

Both $FePO_4$-NPs and non-nano $FePO_4$ are available forms of Fe

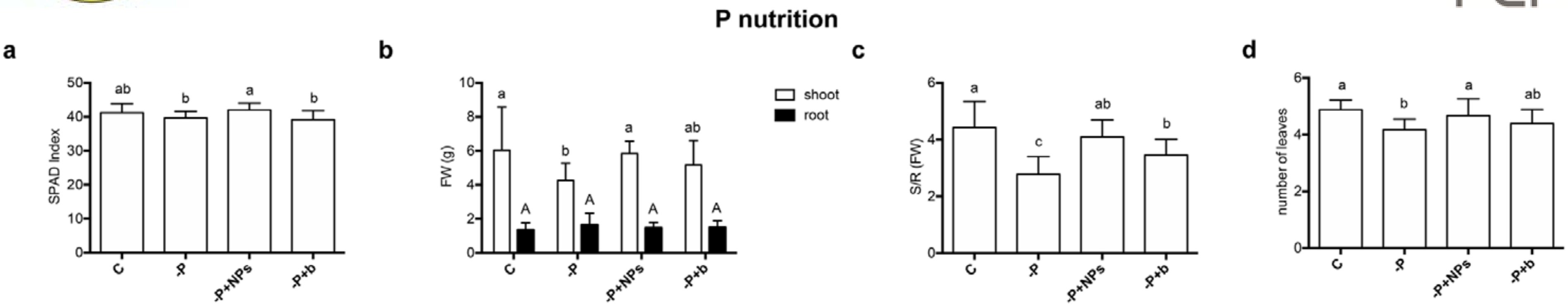


Data are means ± SD of three indipendent experiments with three replicates each (One-way ANOVA with Turkey's post hoc test, $p<0.05$)

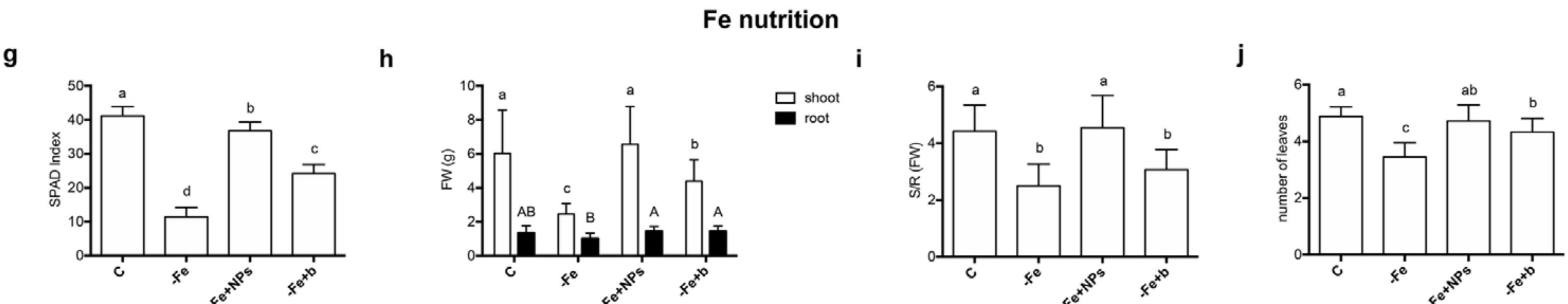
Sega et al., 2020

Effects of $FePO_4$ NPs on maize

Physio-morphological effects



Data are means ± SD of three indipendent experiments with six replicates each (One-way ANOVA with Turkey's post hoc test, $p<0.05$)



Data are means ± SD of three indipendent experiments with six replicates each (One-way ANOVA with Turkey's post hoc test, $p<0.05$)

Plants grown with $FePO_4$-NPs as Fe source show less Fe-deficiency symptoms than plants grown with non-nano $FePO_4$

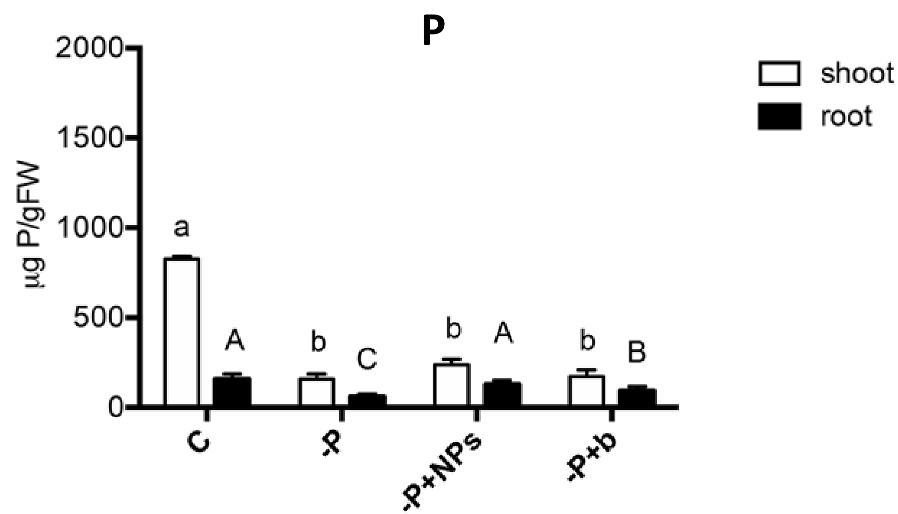
Few symptoms of P deficency could be observed.

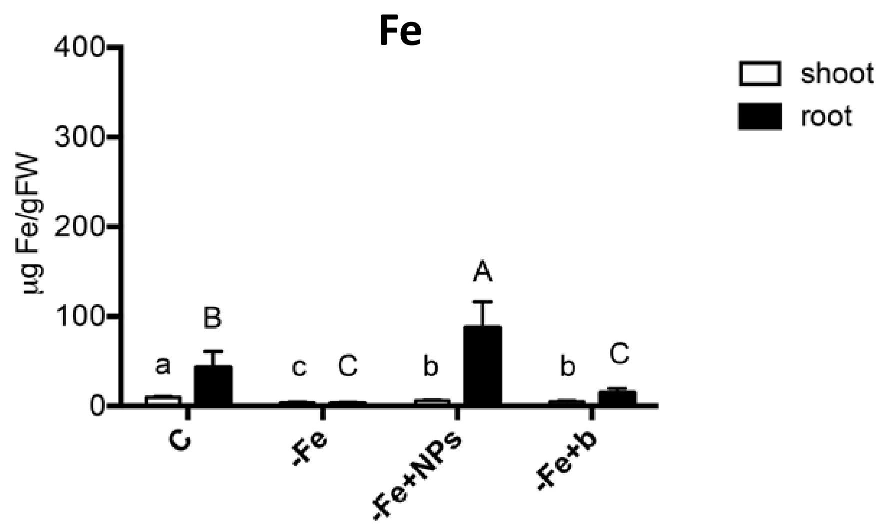
Sega et al., 2020

What about the nutrient content in plant tissues?

$FePO_4$-NPs are not an available P form as the control, but better than non-nano $FePO_4$.

$FePO_4$-NPs are a more available Fe form, than non-nano $FePO_4$.





Data are means ± SD of three indipendent experiments with three replicates each (One-way ANOVA with Turkey's post hoc test, $p<0.05$)

Sega et al., 2020

Effects of $FePO_4$ NPs on plants: a summary

Cucumber

	P				Fe			
	+	-	NPs	bulk	+	-	NPs	bulk
Morphology	xxx	x	xxx	x	xxx	x	xxx	xxx
Nutrient content	xxx	x	xx	x	xxx	x	xxx	xxx

Maize

	P				Fe			
	+	-	NPs	bulk	+	-	NPs	bulk
Morphology	xxxx	xx	xxx	xx	xxxx	x	xxx	xx
Nutrient content	xxxx	x	x	x	xxxx	x	xxx	xx

Safety of $FePO_4$ NPs

NANOTOXICOLOGY, 2017
VOL. 11, NO. 4, 496–506
http://dx.doi.org/10.1080/17435390.2017.1314035

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ORIGINAL ARTICLE

Iron phosphate nanoparticles for food fortification: Biological effects in rats and human cell lines

Lea M. von Moos[a], Mirjam Schneider[a], Florentine M. Hilty[a], Monika Hilbe[b], Myrtha Arnold[a], Nathalie Ziegler[a], Diogo Sales Mato[a], Hans Winkler[c], Mohamed Tarik[d], Christian Ludwig[d,e], Hanspeter Naegeli[c], Wolfgang Langhans[a], Michael B. Zimmermann[a], Shana J. Sturla[a] and Ioannis A. Trantakis[a]

[a]Department of Health Sciences and Technology, ETH Zürich, Switzerland; [b]Institute of Veterinary Pathology, University of Zurich-Vetsuisse, Switzerland; [c]Institute of Pharmacology and Toxicology, University of Zurich-Vetsuisse, Switzerland; [d]Energy and Environment Research Division, Paul Scherrer Institute (PSI), Switzerland; [e]Ecole Polytechnique Fédérale de Lausanne (EPFL), ENAC-IIE, Lausanne, Switzerland

ABSTRACT
Nanotechnology offers new opportunities for providing health benefits in foods. Food fortification with iron phosphate nanoparticles ($FePO_4$ NPs) is a promising new approach to reducing iron deficiency because $FePO_4$ NPs combine high bioavailability with superior sensory performance in difficult to fortify foods. However, their safety remains largely untested. We fed rats for 90 days diets containing $FePO_4$ NPs at doses at which iron sulfate ($FeSO_4$), a commonly used food fortificant, has been shown to induce adverse effects. Feeding did not result in signs of toxicity, including oxidative stress, organ damage, excess iron accumulation in organs or histological changes. These safety data were corroborated by evidence that NPs were taken up by human gastrointestinal cell lines without reducing cell viability or inducing oxidative stress. Our findings suggest $FePO_4$ NPs appear to be as safe for ingestion as $FeSO_4$.

ARTICLE HISTORY
Received 29 October 2016
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KEYWORDS
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