In-vitro Mobilizierung von Kalzium-, Eisen- und Aluminium- Phosphat durch Rhizosphärenbakterien der Afrikanischen Ölpalme

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African oil palm *Elaeis guineensis*

- one of the most important oil crops in the world

juvenil

adult

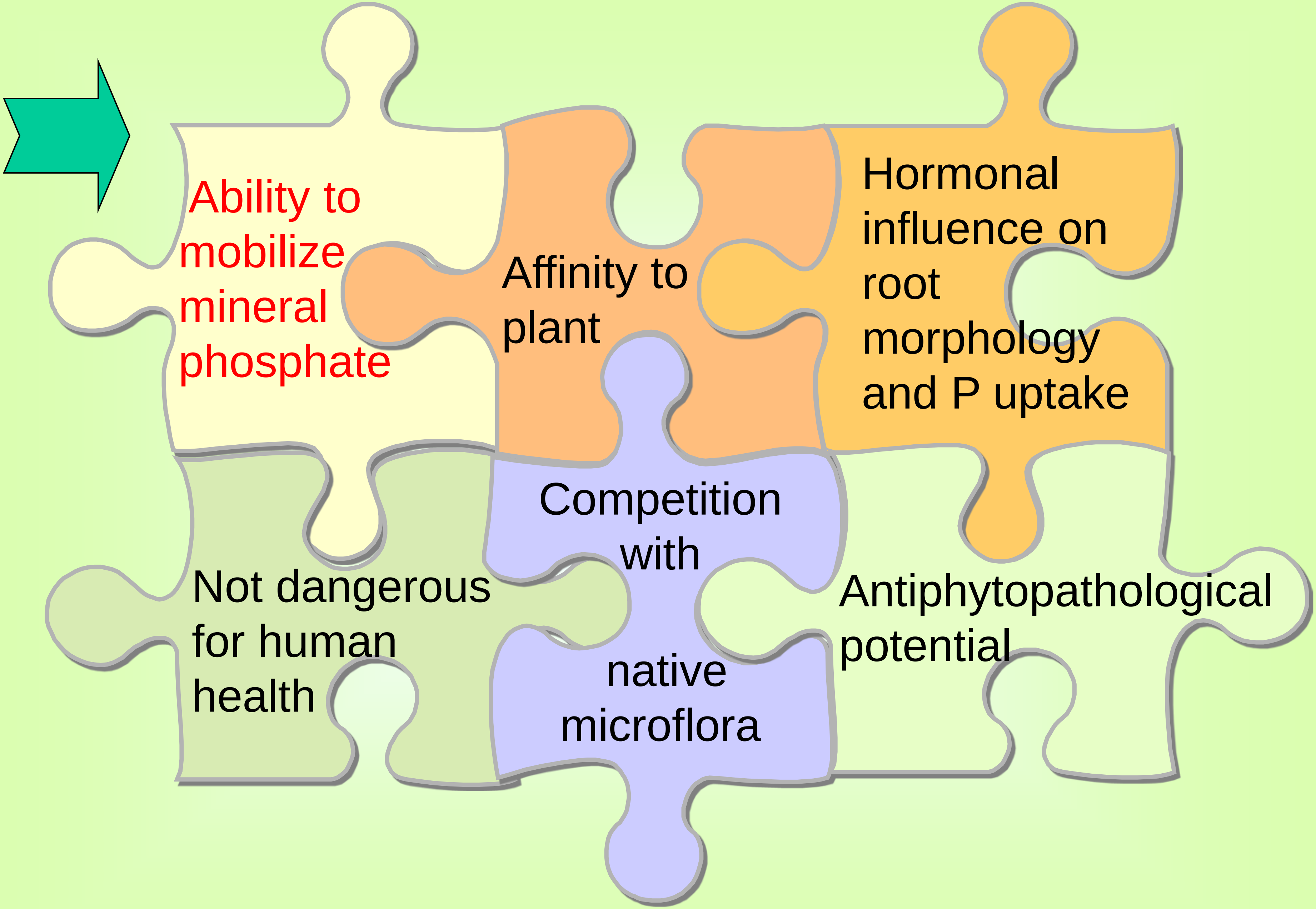
Aim of investigation:

Looking for rhizosphere microbes,which are able to mobilize Ca, Al and Fe from soil for oil palm nutrition

Basis for bio-fertilizer?

Question 1

Which properties are included in potentially biofertilizer strains?


Ability to mobilize mineral phosphate
Affinity to plant
Hormonal influence on root morphology and P uptake
Not dangerous for human health
Competition with native microflora
Antiphytopathological potential

Question 2

Why is ability for phosphate mobilisation important for oil palm ?

Problem

- phosphate supply is one of the most important yield-limiting factors in many tropical and subtropical soils
- high reactivity with Ca oder Fe / Al
- mineral fertilizers hardly available
- mycorrhiza needs 1-4 months to be effective, expensive inoculates

Therefore

→ **Examination of the occurrence of phosphate-mobilising bacteria in the rhizosphere of oil palm**

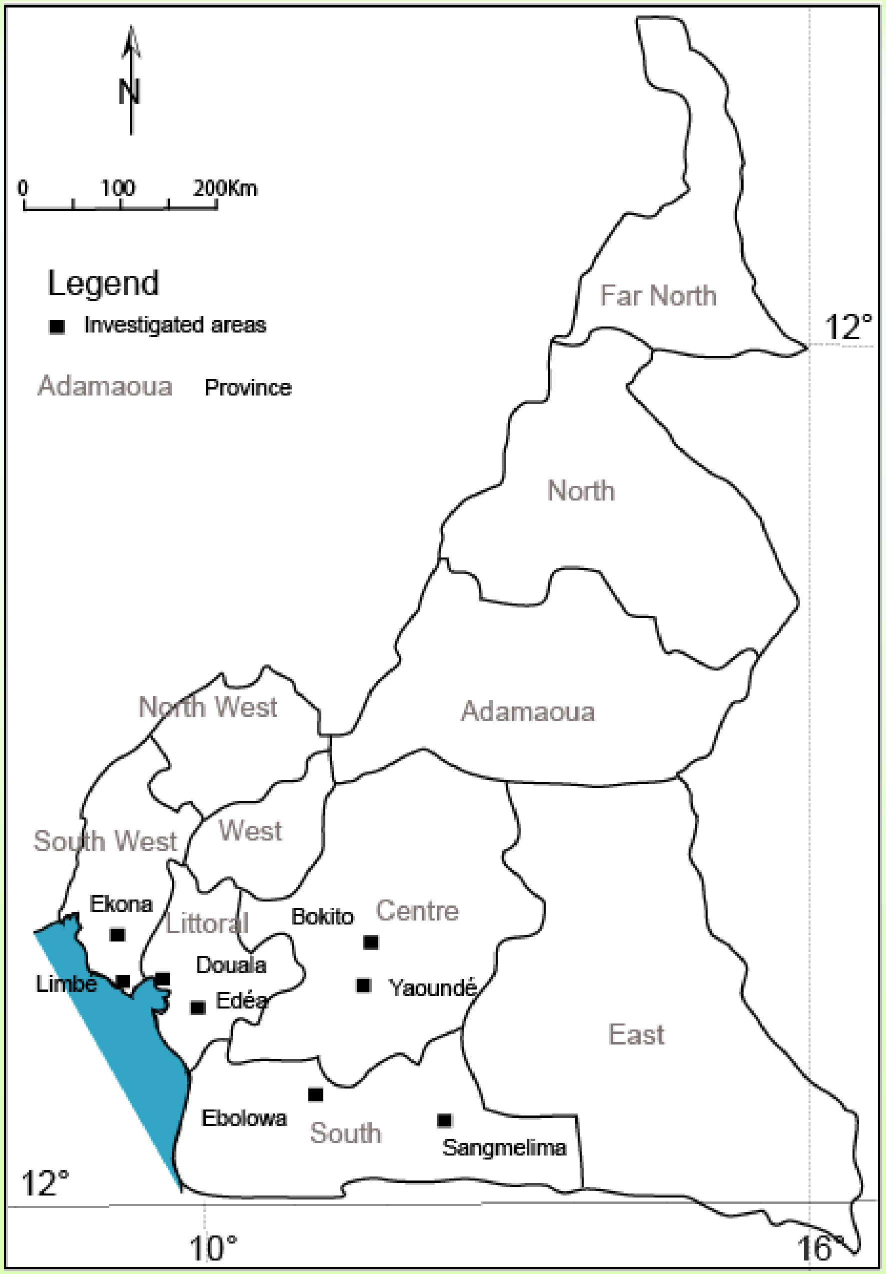
Question ③

How was determined the phosphate mobilization of mineral phosphate by bacteria?

Step 1

- Isolation of bacterial strains from rhizosphere soil (187) and root fragments (90); repeated inoculation for purity and stability

- Fig. Distribution of the investigated areas in humid-forest zones of Cameroon. These areas are located in two main agro-ecological zones of Cameroon:
  a) the humid forest zone with one rainy season and that includes provinces of Littoral and South west
- b) the humid forest zone with two rainy seasons that consists of Centre, South and East provinces. These areas represent the main oil palm plantations.

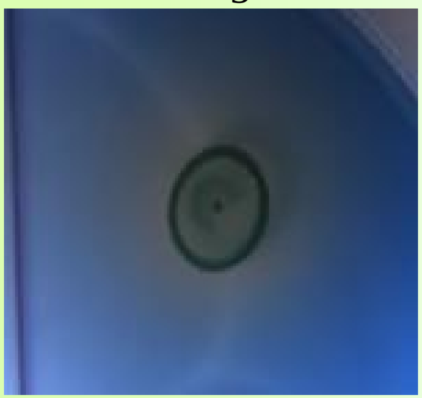


Step 2

•Qualitative test on ability to solubilize $Ca_3(PO_4)_2$, $FePO_4$ und $AlPO_4$ using agarplates with dye(bromo cresol green); evaluation of halo (lysing) zones

1-EMJ_5-Fe-P

2-EDJ_8-Fe-P



3-EDA_2-Al-P

4-DA_9-Al-P

5-EA_4-Ca-P

6-EMR_5-Ca-P

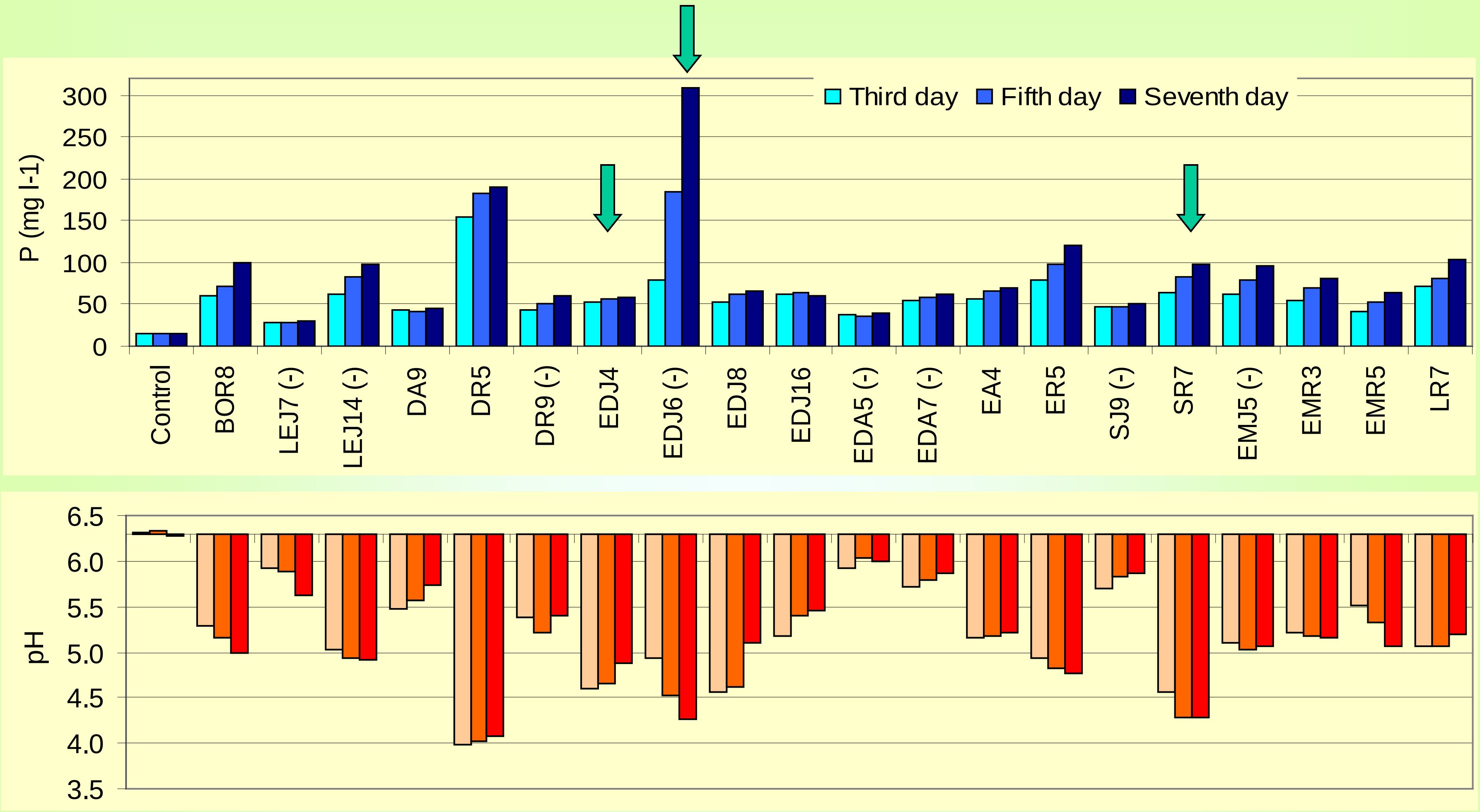
Figure: (1-6): Halo zones surrounding colonies on five days agar plates culture containing $FePO_4 \cdot 2H_2O$ (1 - 2), $AlPO_4 \cdot H_2O$ (3 - 4) and $Ca_3(PO_4)_2$ (5 - 6). Criterion for pre-selection: diameter of colonies and lysing zones

Step 3

•Quantitative determination of P release fom $Ca_3(PO_4)_2$, $FePO_4$ or $AlPO_4$ in liquid culture. Soluble P was determined by colometry

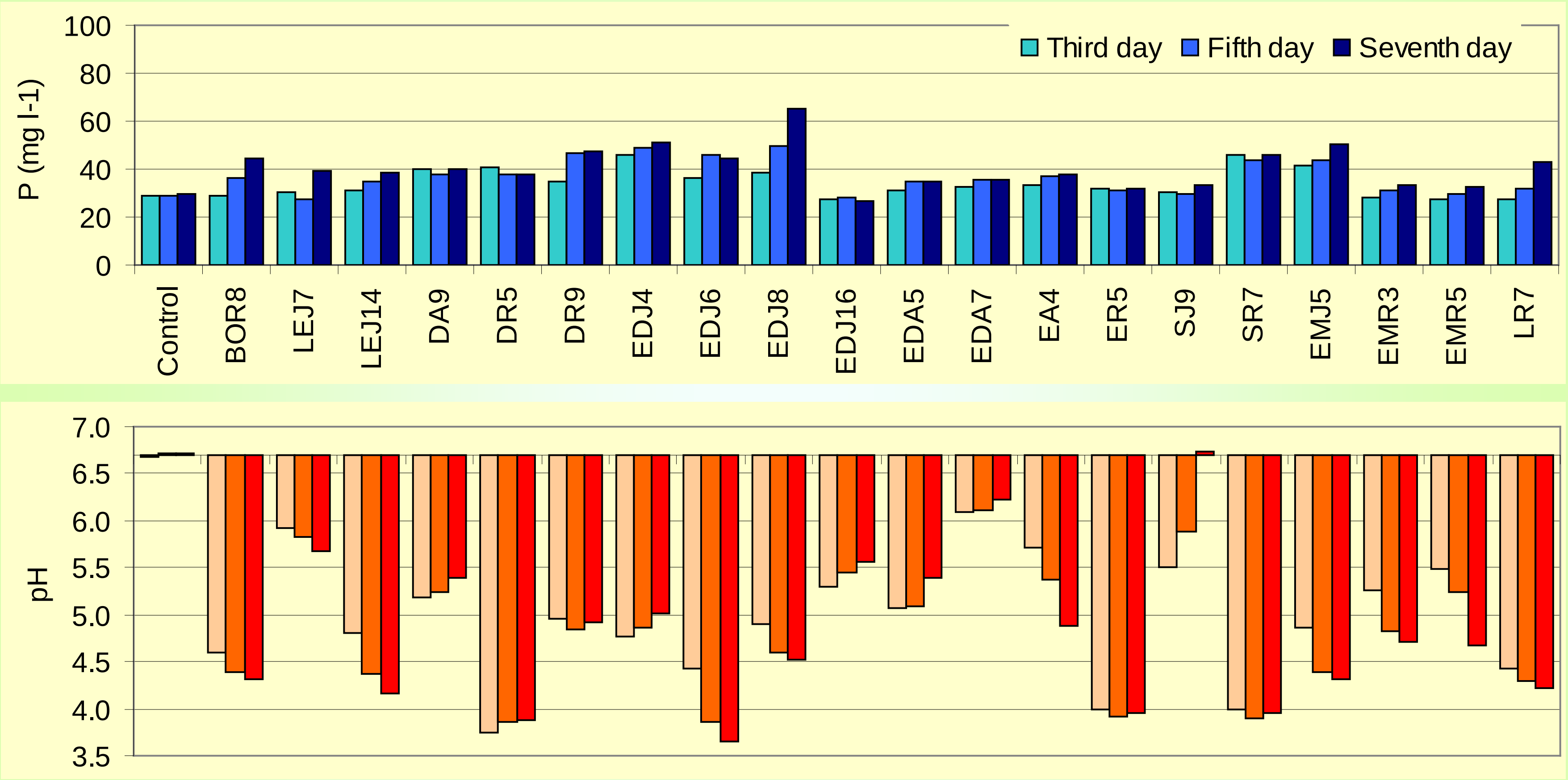
20 selected strains are proved (for comparison also strains without halo zones)

$Ca_3(PO_4)_2$



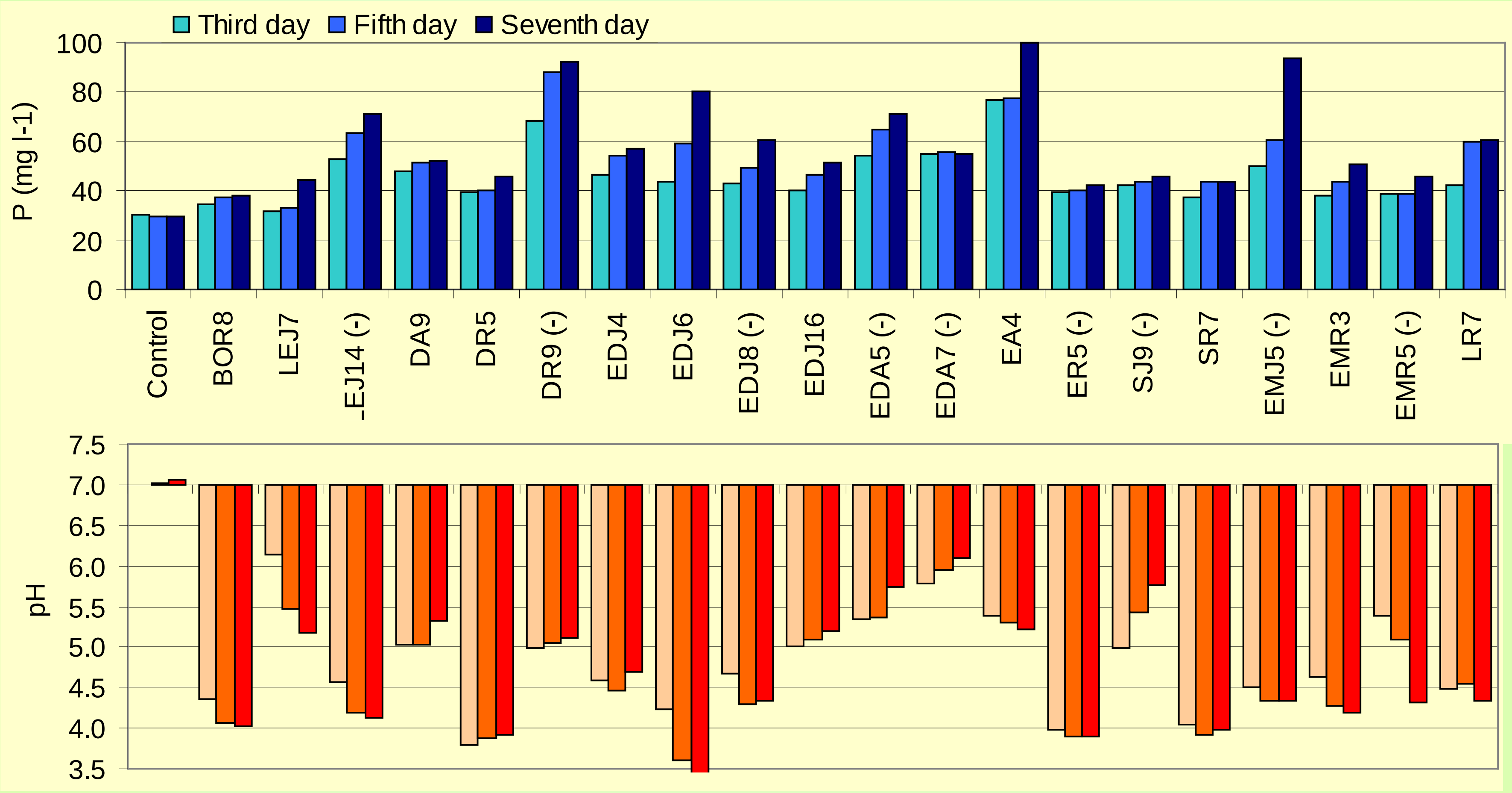
- **P releasing was correlated –as a rule –with ph decrease, but there were differences between both properties (see arrows)**

$FePO_4 \cdot 2\ H_2O$



• **Only some strains mobilized P, despite strong pH decreases**

$AlPO_4 \cdot H_2O$

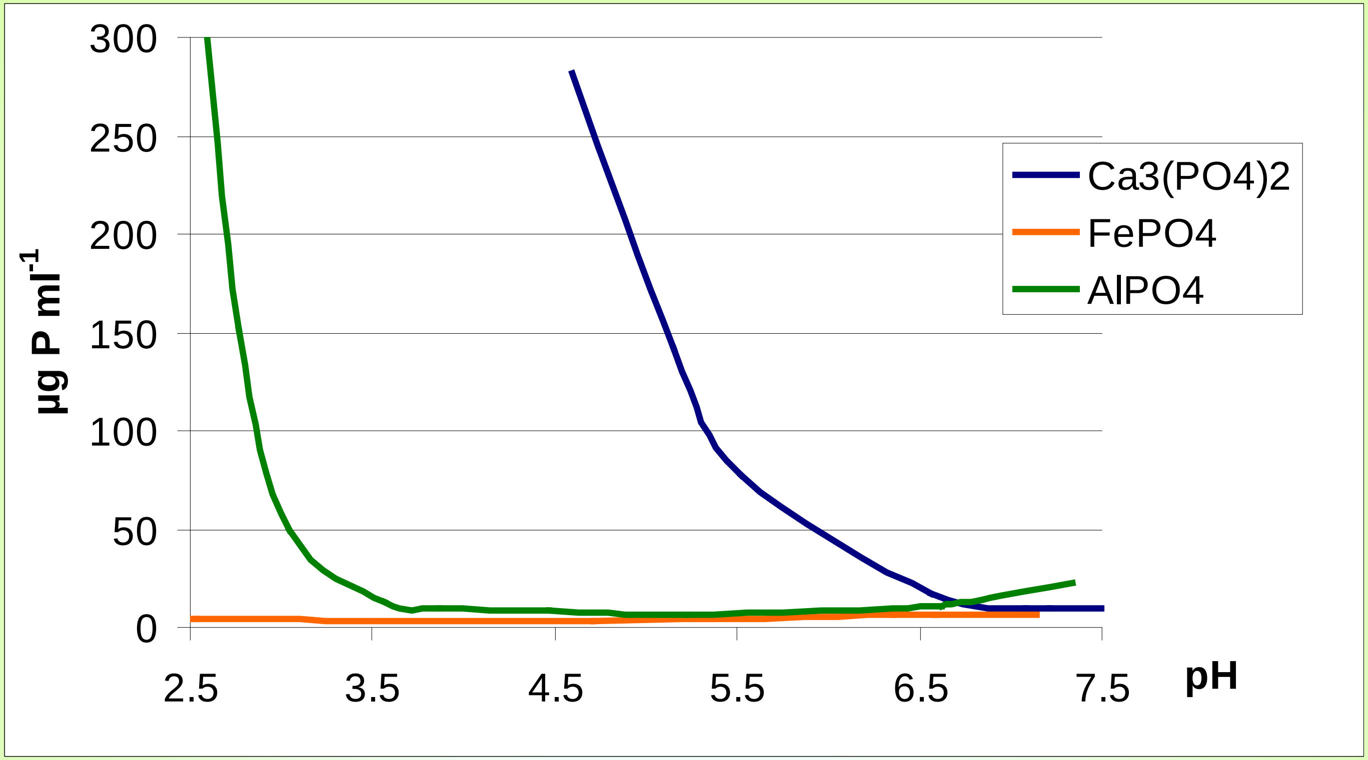


- All strains released P, but no correlation with pH decrease

Question ④

- How does the pH affect the solubility of these phosphates?

To prove this question, the 3 phosphates were extracted with HCL and NaOH of different concentrations

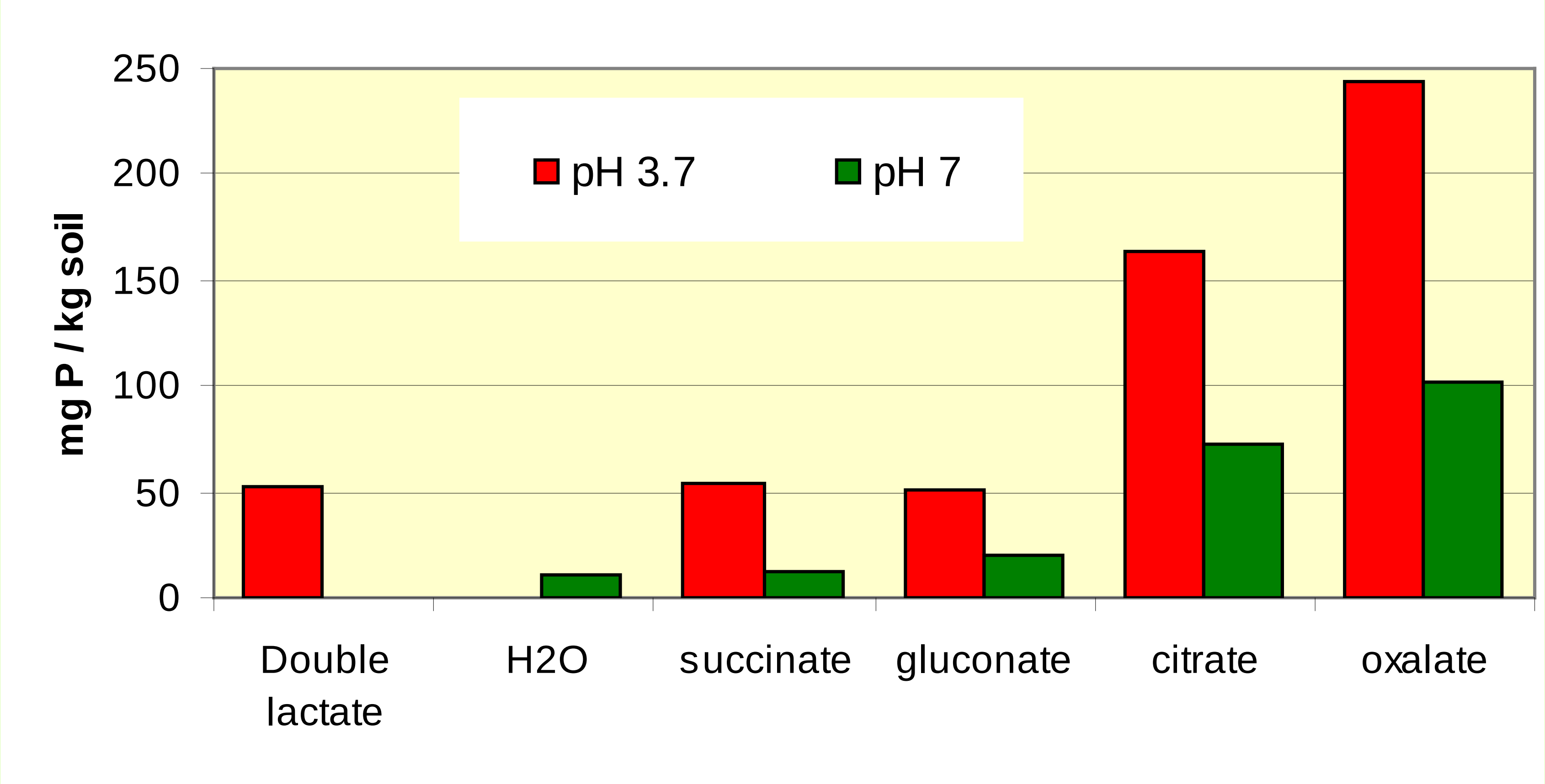


- CaP solubility increases exponentially with decreasing pH (can explain P release in vitro partly)
- AlP only solubilised at pH levels lower than 3.5 (impossible in bacterial cultures and not useful in plant rhizosphere; could explain P release with some fungal strains)
- Acidification no explanation for FeP solubilisation :not soluble at all pH

Question ⑤

- Are carboxylic acids (partly) responsible for P solubilisation? Which carboxylic acids solubilise P under natural soil-pH conditions and in vitro?

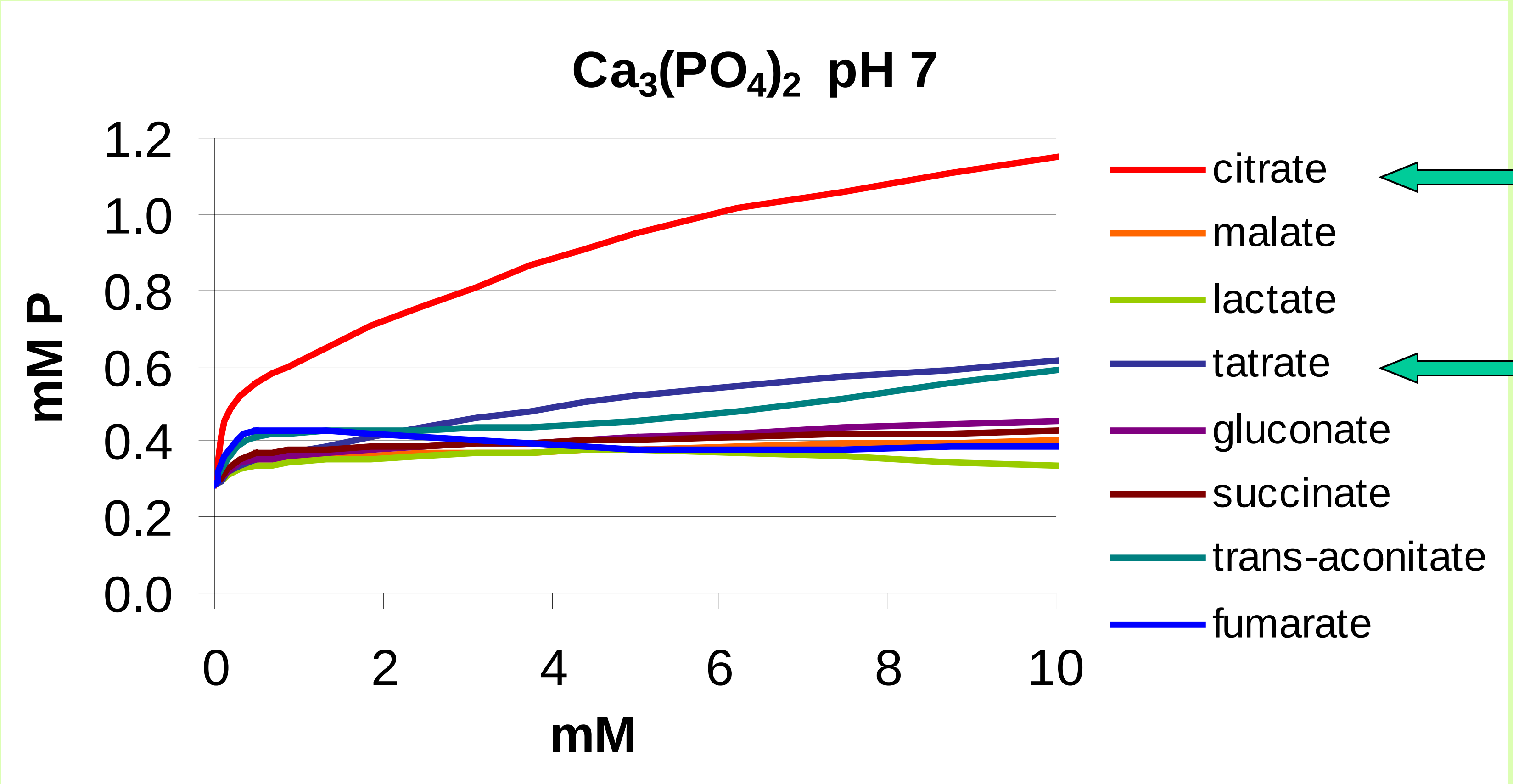
Soil conditions



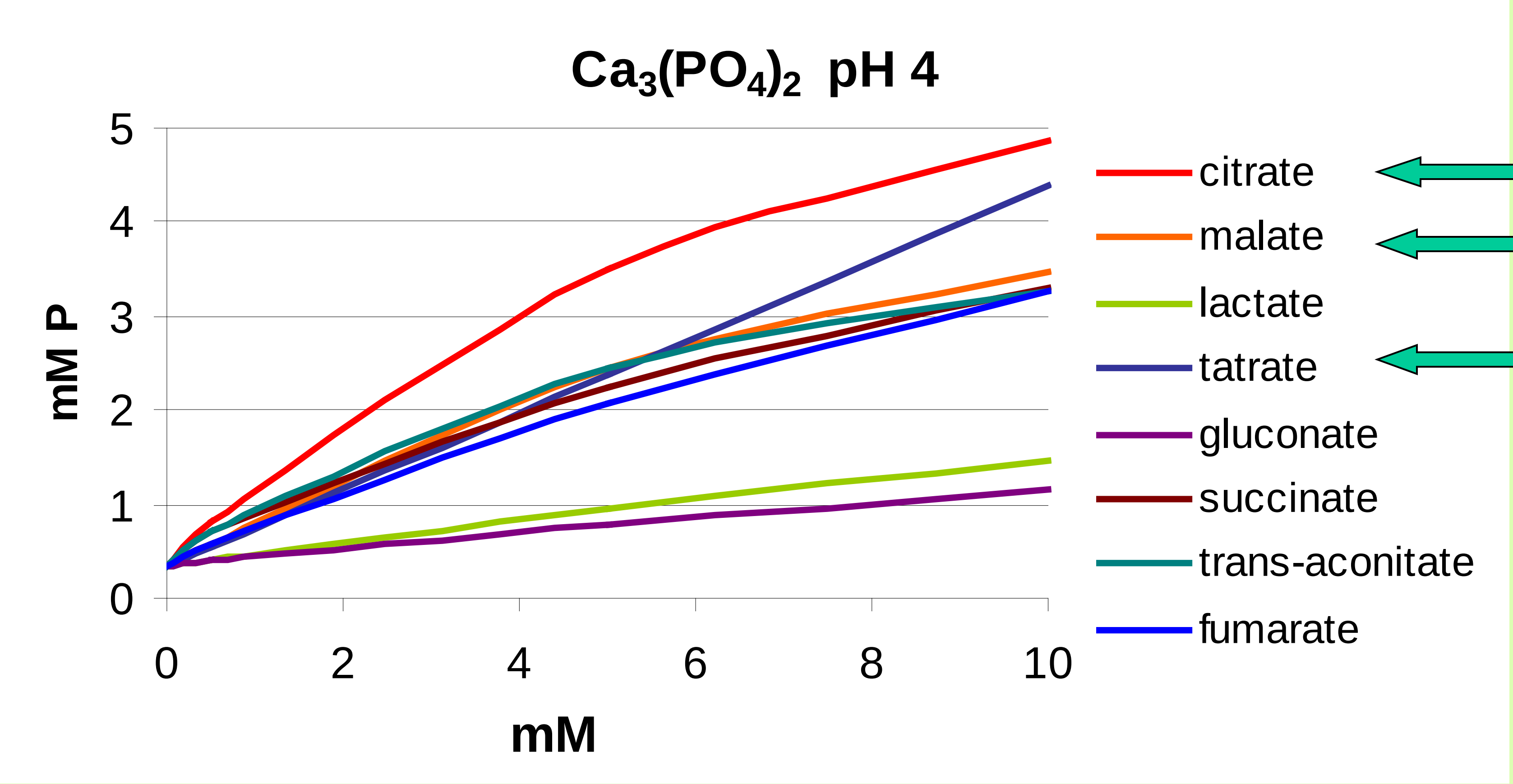
(90 min extraction of 5 g soil with 250 ml solution containing 5 g l^{-1} of different substances, average from 10 different soils) Carboxylic acids were able to more P than double lactate, esp. at pH 3.7

Next step therefore:

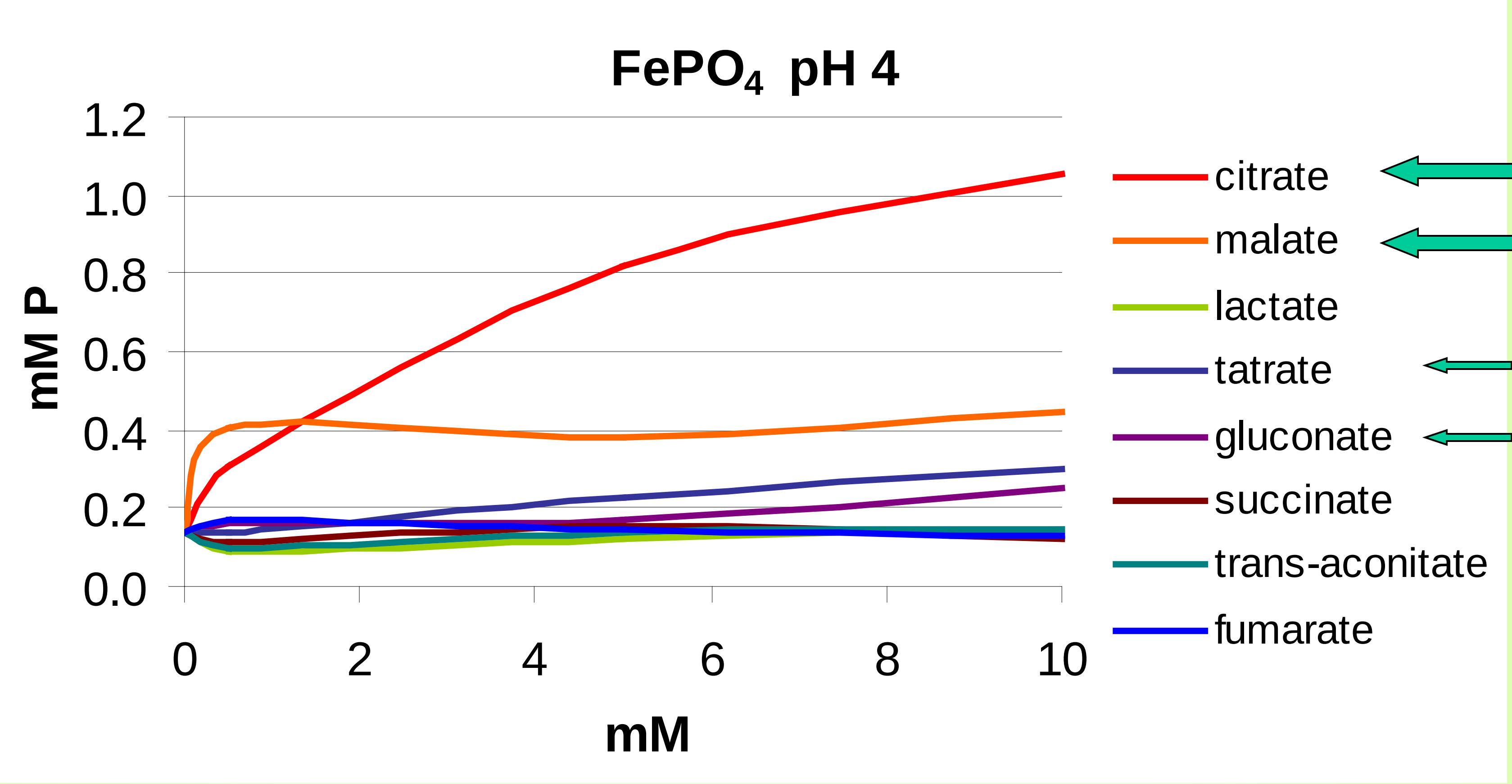
In vitro examination of different carboxylic acids for solubilization of Ca-, Fe- and Al phosphates



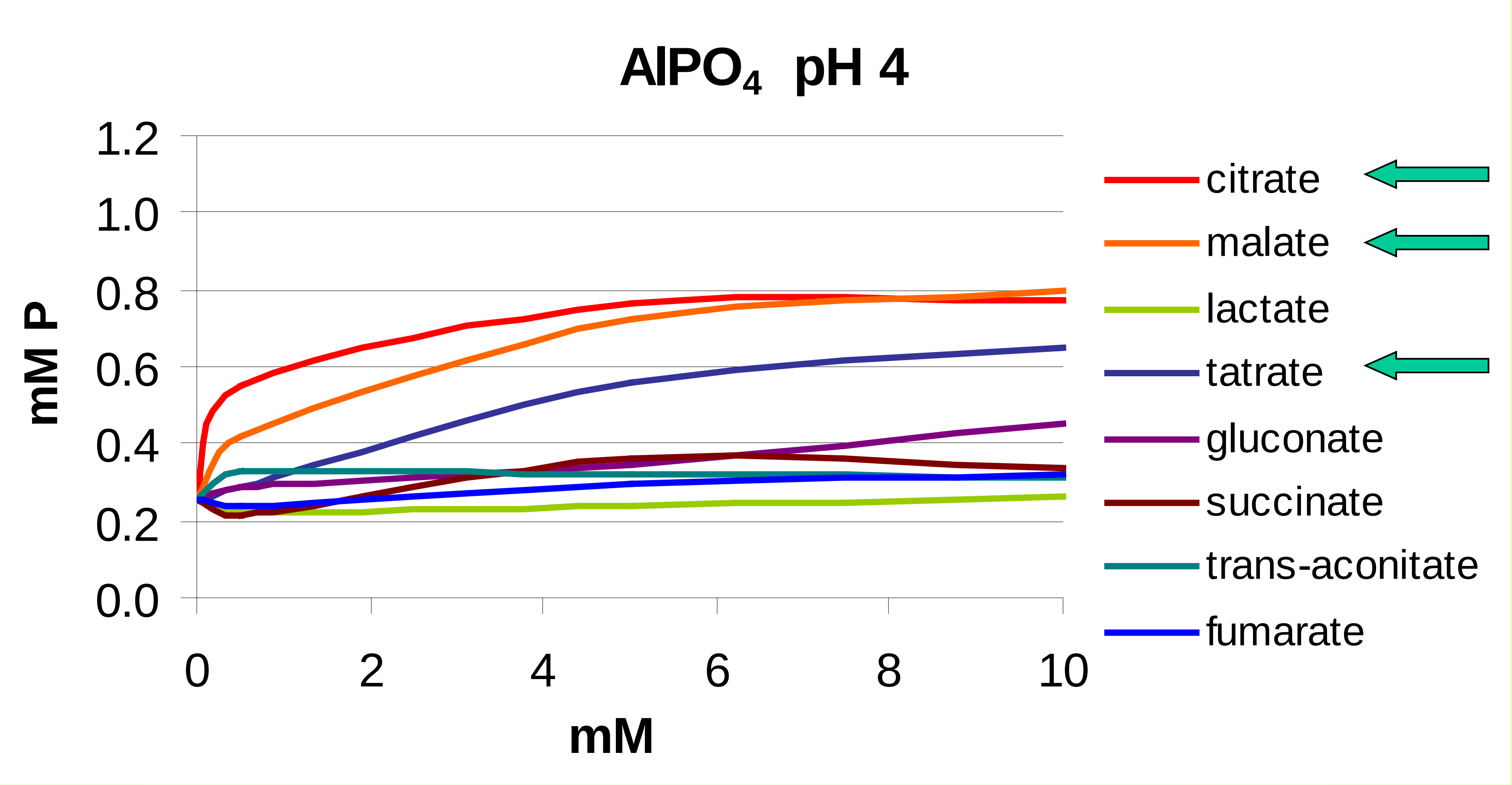
At pH 7: Only citrate increased CaP solubility remarkably



At pH 4 (like in bacterial cultures) some acids increase CaP releasing and complete pH effect. **But**: sparingly soluble CaP occurs in neutral or alkaline soils



Iron phosphate was mobilised by citrate and malate, in a smaller extend by tartrate and gluconate



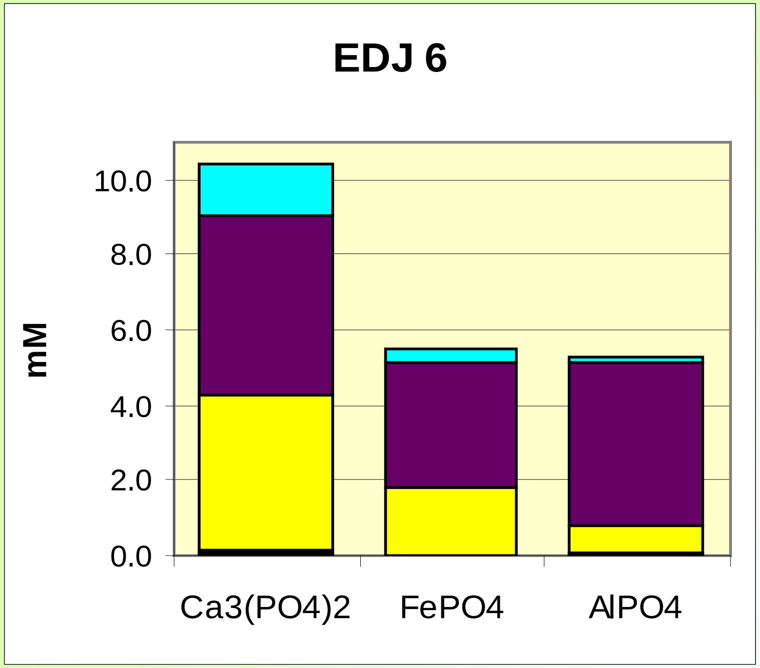
AlP by citrate, malate, tartrate > gluconate.

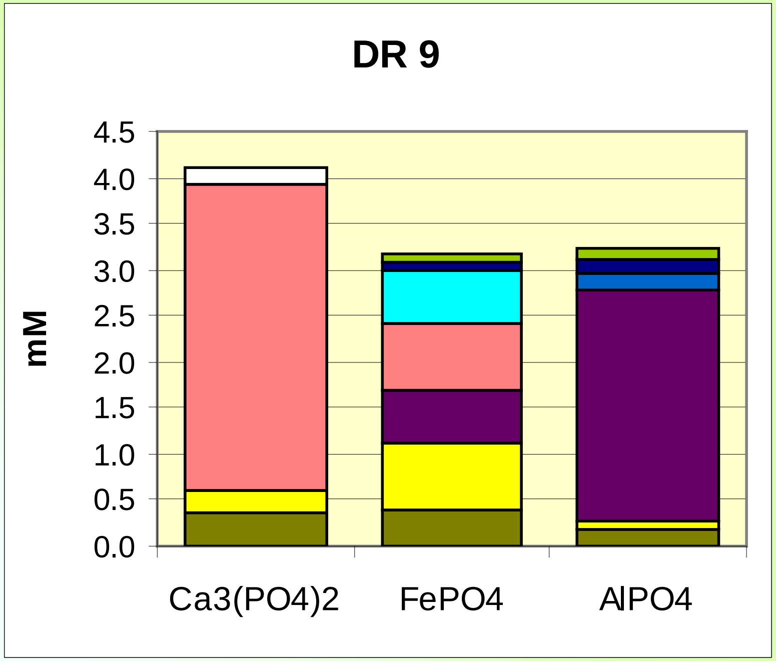
Acidification no explaination for P solubisation

Question ⑥

- Which carboxylic acids were produced by bacterial strains and influenced the P releasing?

- This seems important in case of Al or Fe phosphates (no or low pH influence of pH value) (no halo zone) (halo zone)





mg P/l 320 45 80 7d 42 40 50

oxalate	trans-aconitate	oxalacetate	citrate
tartrate	gluconate	xylonate	malate
succinate	lactate	fumarate	

Strains produced lot of carboxylic acids and mobilized P. EDJ 6 = most efficient CaP mobilizer: High citrate production and missing halo zone (precipitation of Ca citrate in agar plate test? Limit for pre-selection at agarplates.Substitution by citrate test?). Citrate, tartrate, gluconate most effective.

Conclusions

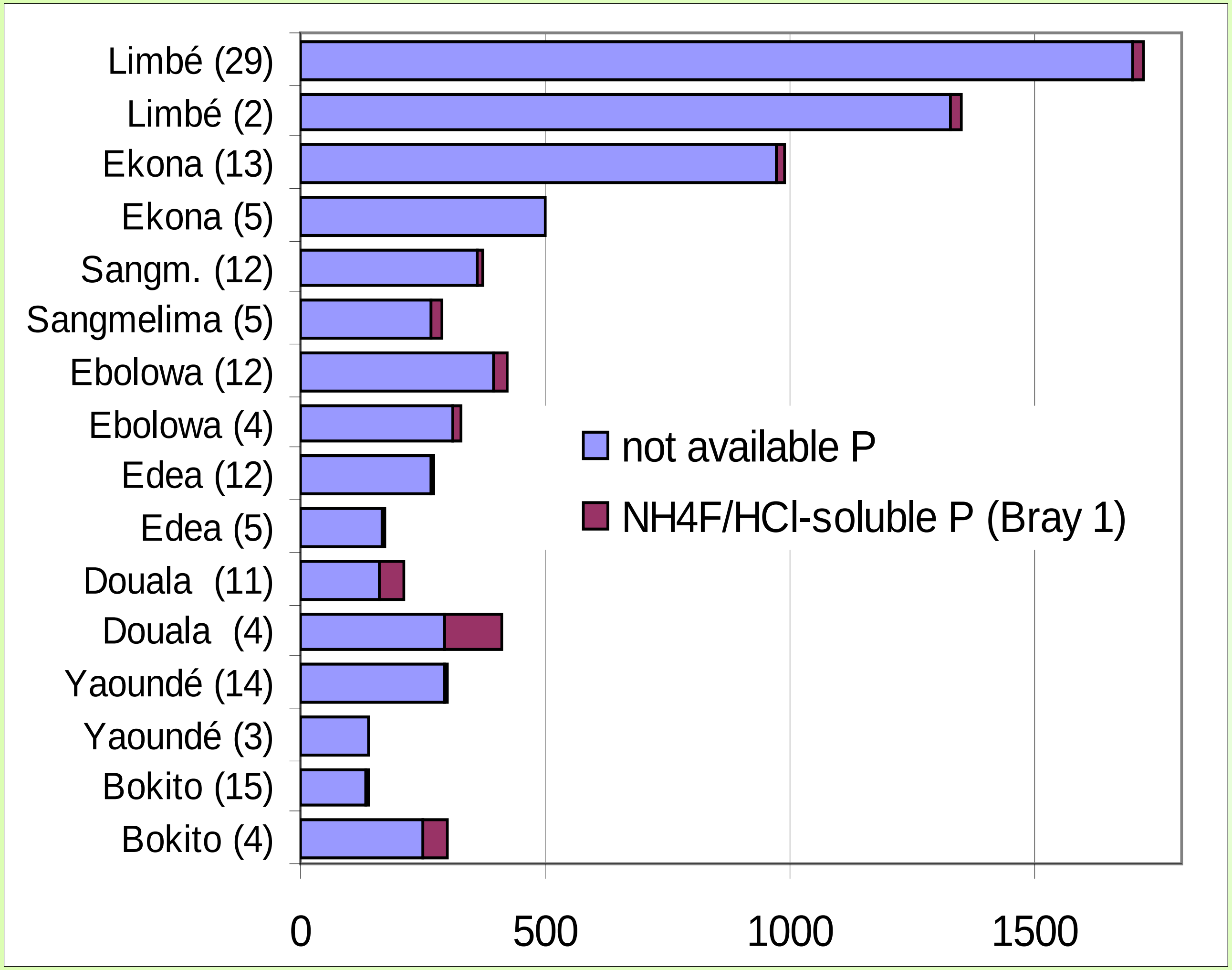
- P-solubilizing bacteria occur in the rhizosphere of *Elaeis guineensis*; role of microbial P mobilization can be assumed
- $Ca_3(PO_4)_2$ -solubilization *in vitro* by acidification and different carboxylic acids; at pH 7 (natural soil conditions) mainly by citrate
- $FePO_4$ and $AlPO_4$ solubilization by citrate, malate, tartrate, (gluconate)
- Citrate can prevent halo zones by precipitation of Ca-citrate!
- Screening for citrate production would improve the selection of potential P-mobilizing microorganisms

Thank you to all the colleagues

First of all:

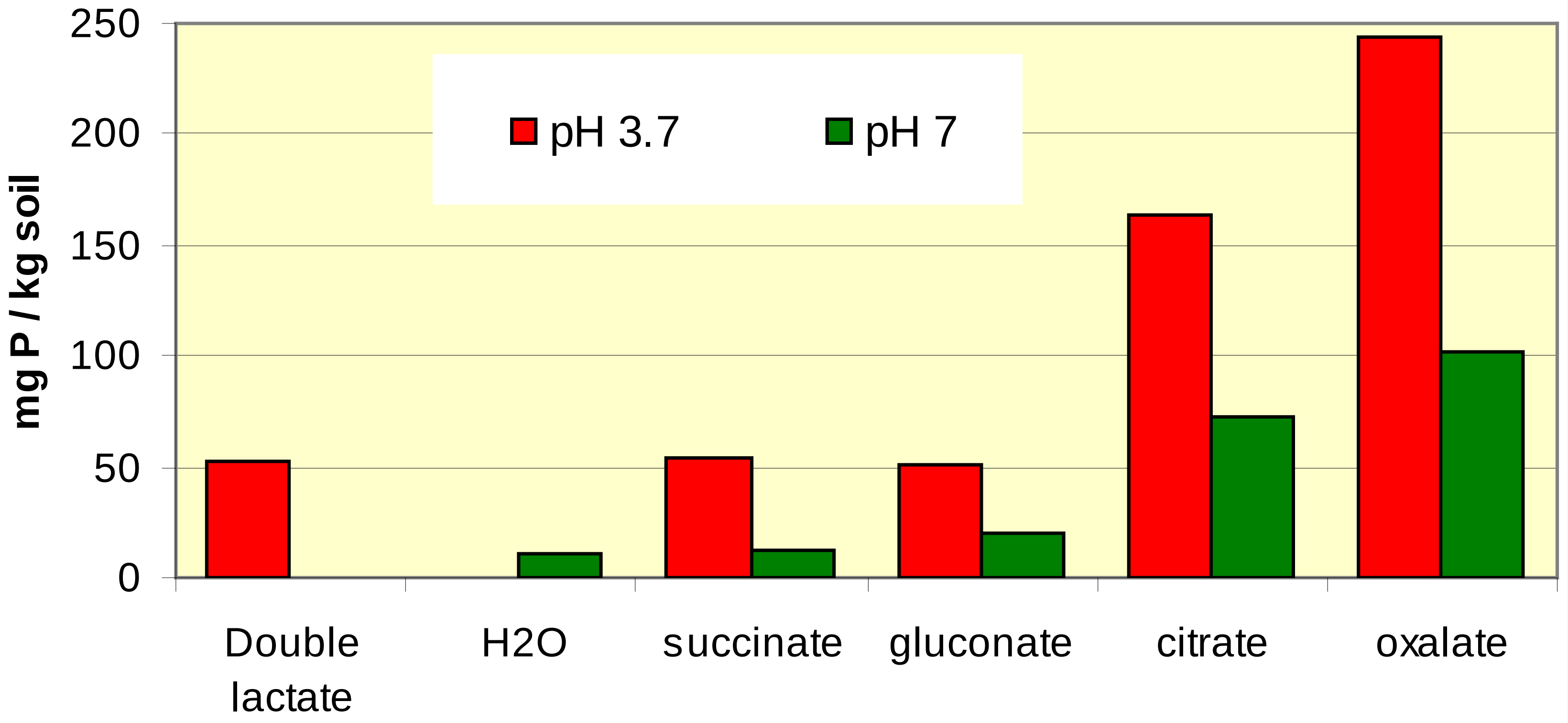
Dr.Henri Fankem, Cameroon

P concentration in soils (mg kg^{-1})

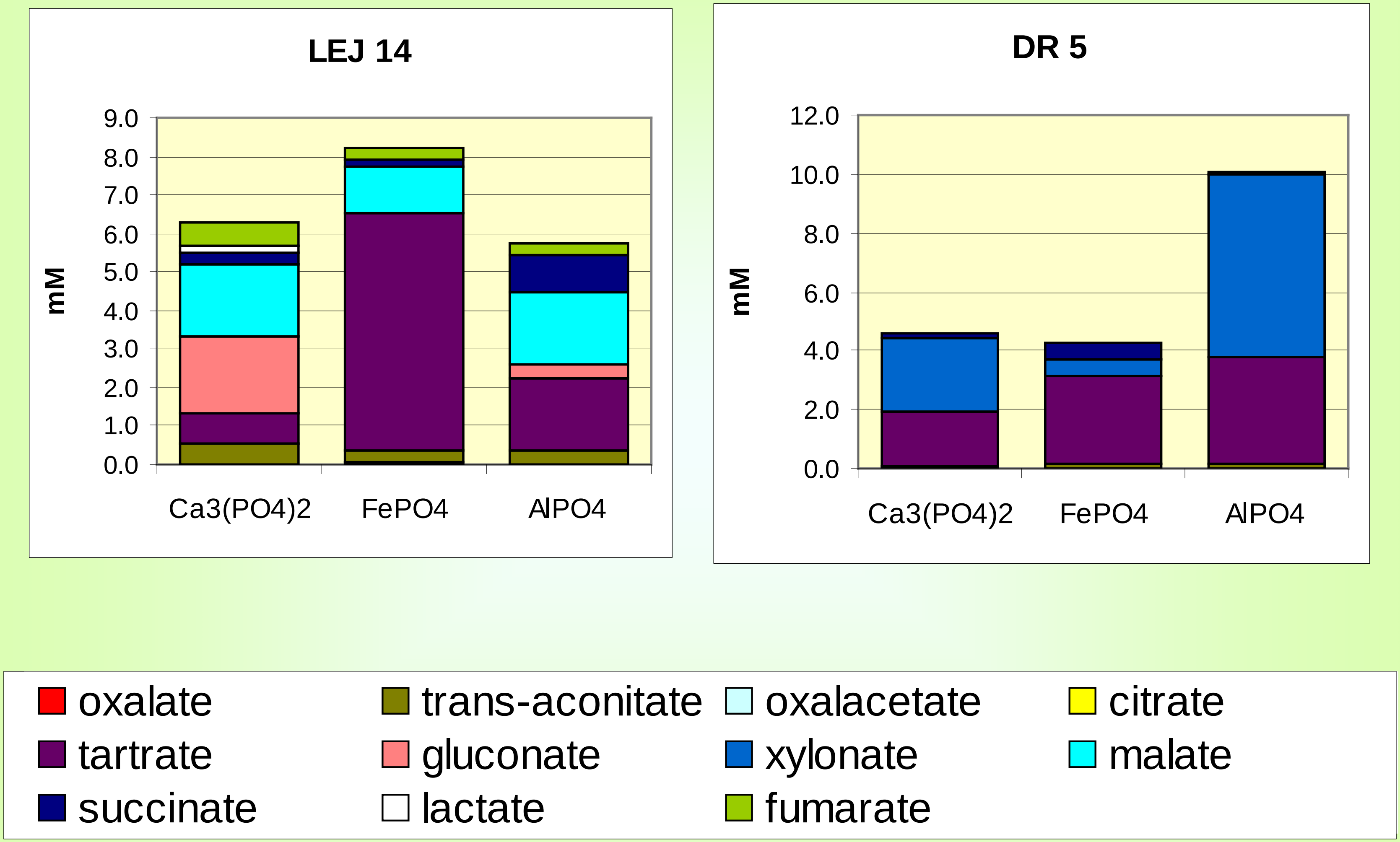


pH	% Fe	% Al
6.0	9.4	6.1
4.8	10.6	5.2
5.2	8.9	5.3
4.3	11.2	5.5
4.0	4.6	5.3
3.7	4.5	5.4
4.1	3.1	4.5
4.0	2.6	4.1
4.9	7.0	3.5
4.1	2.1	3.1
4.5	0.8	1.0
4.8	1.0	1.4
5.0	5.2	4.5
5.5	0.9	1.1
4.7	1.2	1.3
7.4	1.4	1.8

Soil conditions

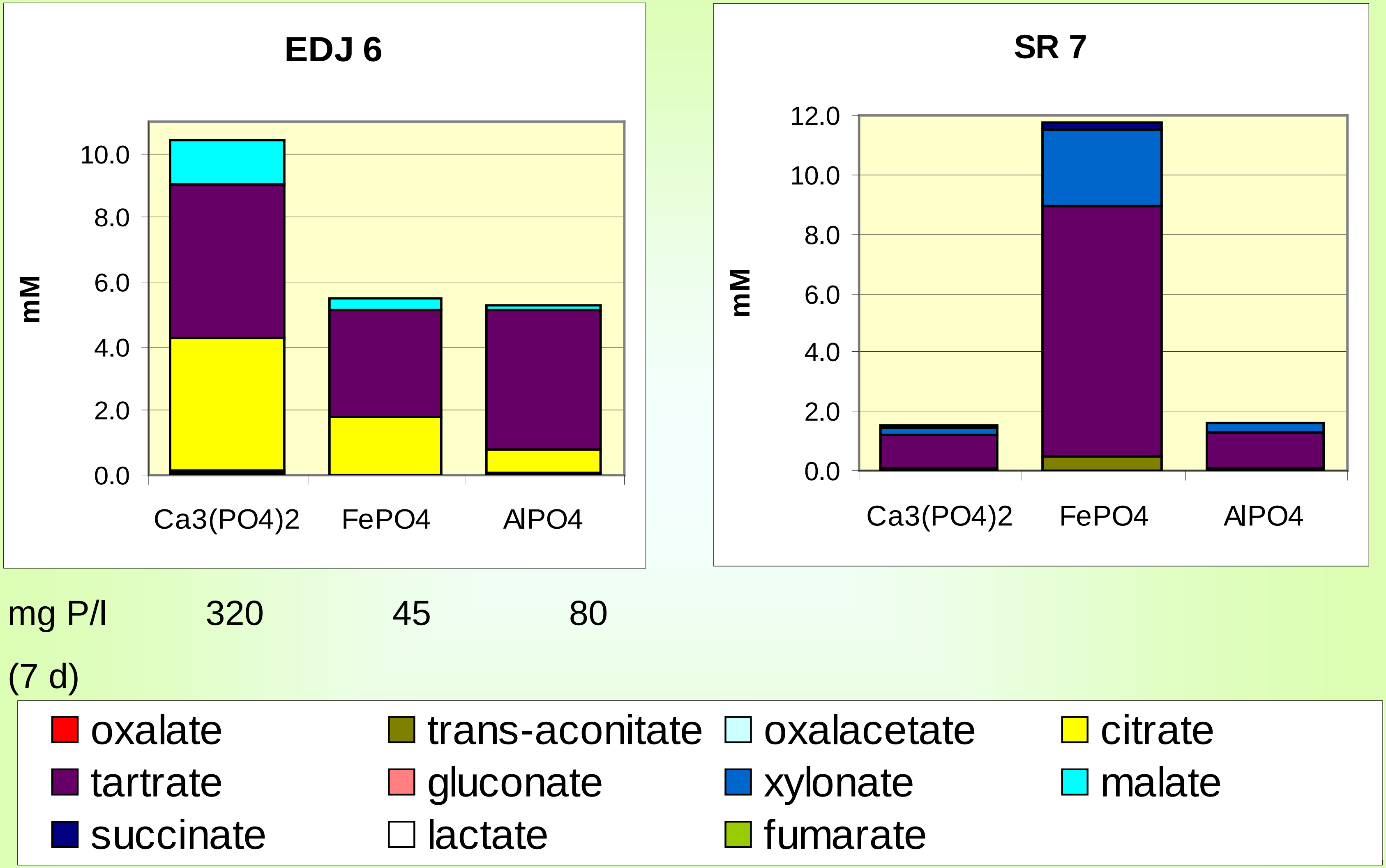


(90 min extraction of 5 g soil with 250 ml solution containing 5 g l^{-1} of different substances, average from 10 different soils)

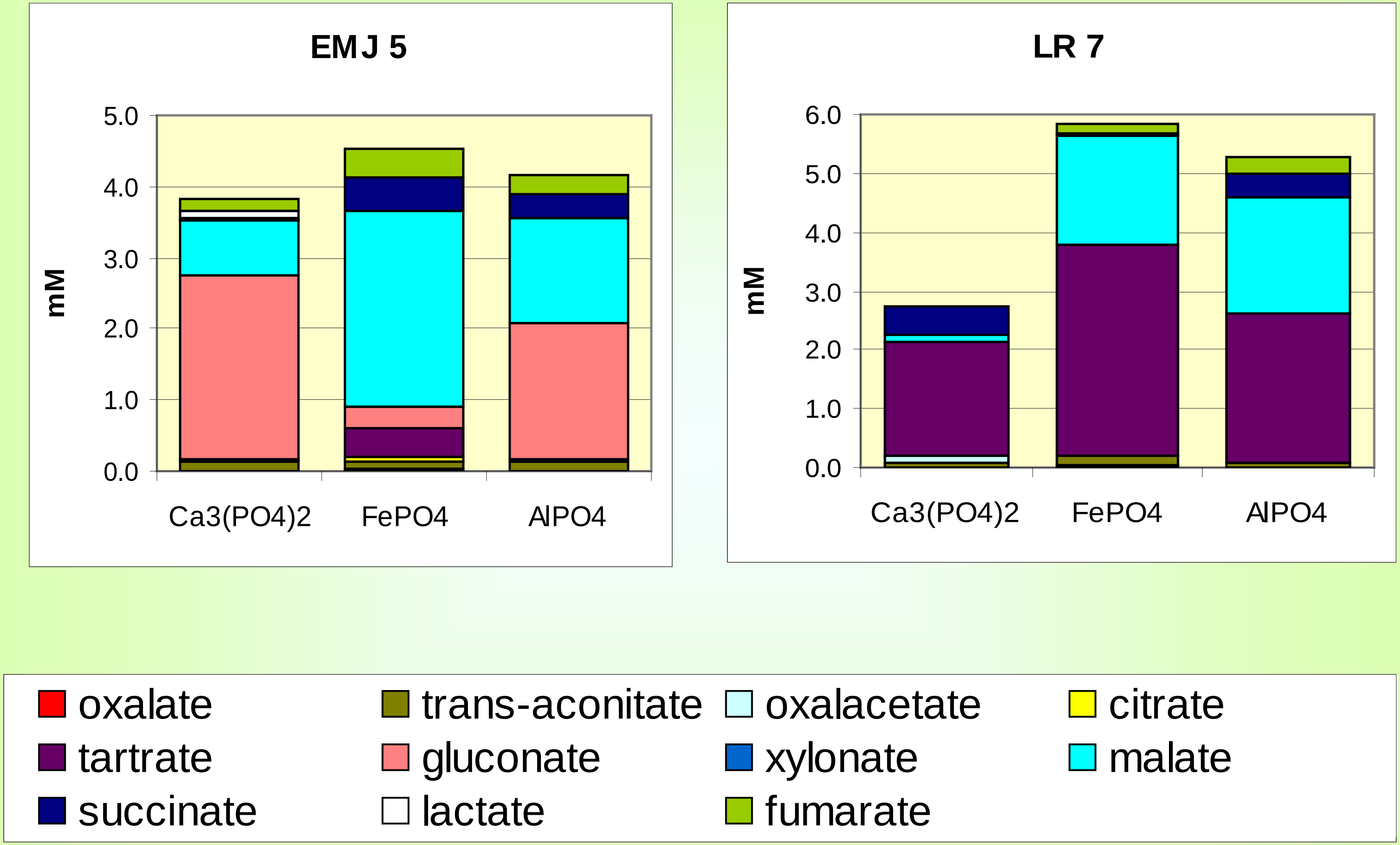


LEJ 14
mM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Ca3(PO4)2
FePO4
AlPO4
DR 5
mM
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Ca3(PO4)2
FePO4
AlPO4
oxalate
trans-aconitate
oxalacetate
citrate
tartrate
gluconate
xylonate
malate
succinate
lactate
fumarate

(no halo zone) (halo zone)

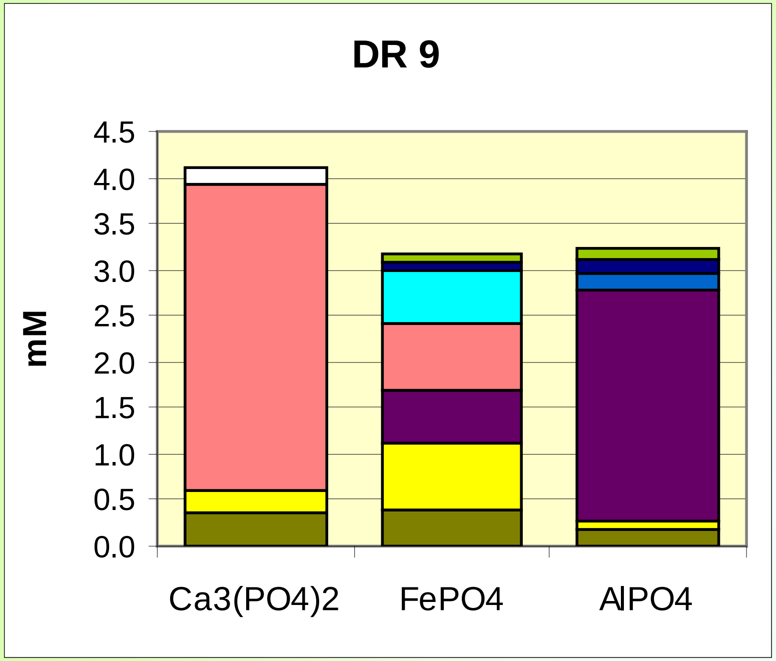


Strain EDJ 6 = most efficient CaP mobiliser: High citrate production and missing halo zone (precipitation of Ca citrate in agar plate test?)

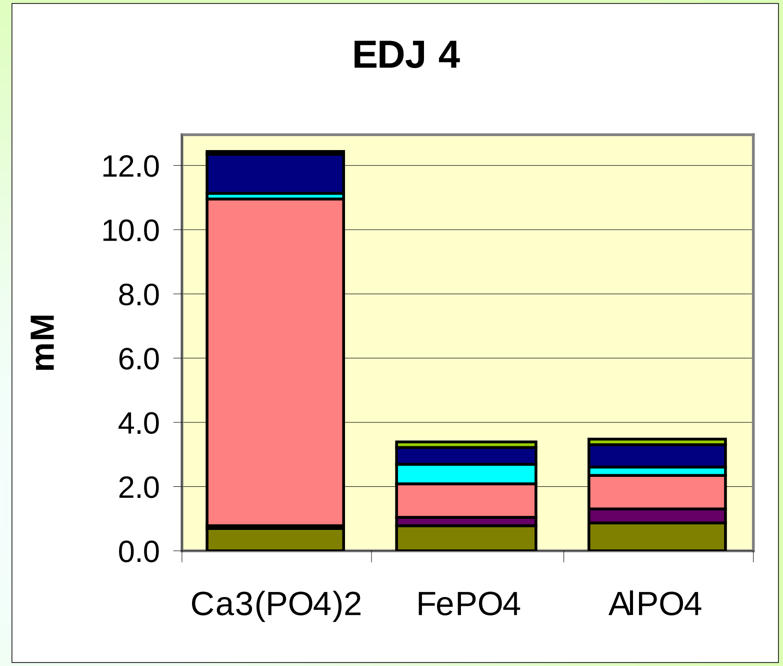


EMJ 5
mM
5.0
4.0
3.0
2.0
1.0
0.0
Ca3(PO4)2
FePO4
AlPO4
LR 7
mM
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Ca3(PO4)2
FePO4
AlPO4
oxalate
trans-aconitate
oxalacetate
citrate
tartrate
gluconate
xylonate
malate
succinate
lactate
fumarate

(halo zone)



(halo zone)



mg P/l	42	40	50
(7 d)			

oxalate	trans-aconitate	oxalacetate	citrate
tartrate	gluconate	xylonate	malate
succinate	lactate	fumarate	