



HOKKAIDO
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Investigation into determination method of anti-malarial drugs in agricultural soil and plant assumed as human urine reuse

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sustainable sanitation
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photo by N. Hijikata

Back ground



The use of urine for agriculture

Advantage

- ◆ It's effective to improve sanitation system
- ◆ Urine contains nitrogen, phosphorus and potassium

Disadvantage

- ◆ **Urine contains pharmaceuticals**
- ◆ Salt pollution

Problems of pharmaceuticals

Germination and young plant growth was inhibited by pharmaceuticals (Jjemba, 2002)

Bacterial resistance have been increased because of pharmaceuticals spread in environment (Harnisz *et al.*, 2011)

Some pharmaceuticals has potential to be taken up into plant (Winker *et al.*, 2010)

Back ground

- ◆ African country or South Asia are target area to use urine
- ◆ In these area, Malaria is common disease
- ◆ More than 300 million people are infected with malaria
- ◆ In Burkina-Faso, 4000 kg of anti-malarial drugs are consumed every year




Anti-malarial drugs	Unchanged Urine excretion	MW	LogKow	pKa
Chloroquine	43.5%	320	4.3	10.1
Doxycycline	40.0%	444	-1.36	3.09
Quinine	13.0%	324	2.6	8.7
Artesunate	-	384	2.5	3.74
Pyrimethamine	0.3%	249	2.41	7.34
Sulfadoxine	-	310	-0.24	6.3
Lumefantrine	-	529	8.67	-
Mefloquine	9.0%	378	3.85	8.6

Objectives

If we use urine, we should research the fate of pharmaceuticals


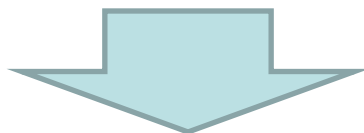
- ◆ Uptake by plant ?
- ◆ Remain in soil ?
- ◆ Decomposed ?

*However, nobody knows the fate of anti-malarial drugs
Even the method for determination*



Investigation of method for determination of
anti-malarial drugs in **soil**

Investigation of method for determination of
anti-malarial drugs in **plant**



Which anti-malarial drugs have the potential
to be taken up into plant ?

Materials & Methods

The method for determination from soil and plant



10 g soil sample
1 g plant sample



Water
Acetone
Methanol
Methanol + KCl



Add 30ml extraction solvent

Shaking extraction 15min

Sonication 10min

Centrifugation 6000rpm 5min

× 2



Diluted 100 times
Adjusted pH = 2.00 ± 0.05



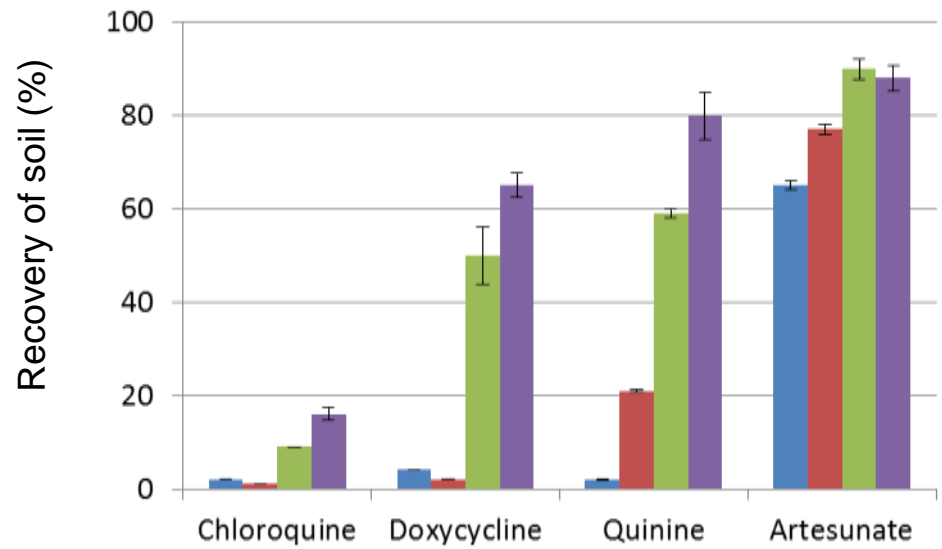
Eluted with 5ml Acetonitrile +
5ml Acetonitrile : Water = 1:1



Determined by LC/MS



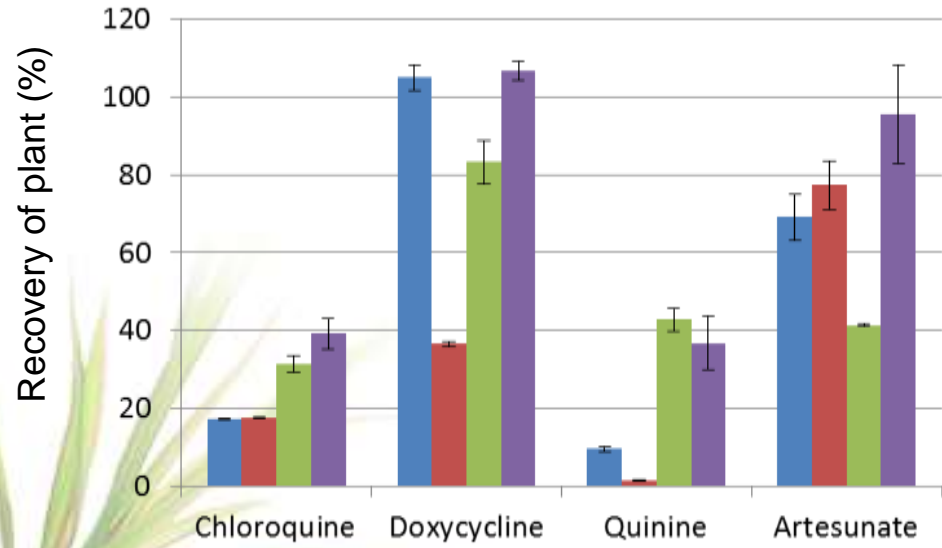
Results ~recovery of soil and plant~



	Recovery by Methanol + KCl
Pyrimethamine	58 %
Sulfadoxine	95 %
Lumefantrine	57 %
Mefloquine	98 %

- Add water
- Add acetone
- Add Methanol
- Add Methanol + KCl

Mixture of Methanol and KCl was most effective



- Add water
- Add acetone
- Add Methanol
- Add Methanol + KCl


	Recovery by Methanol + KCl
Pyrimethamine	45 %
Sulfadoxine	94 %
Lumefantrine	22 %
Mefloquine	88 %

Objectives

If we use urine, we should research the fate of pharmaceuticals


- ◆ Uptake by plant ?
- ◆ Remain in soil ?
- ◆ Decomposed ?

*However, nobody knows the fate of anti-malarial drugs
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Which anti-malarial drugs have the potential
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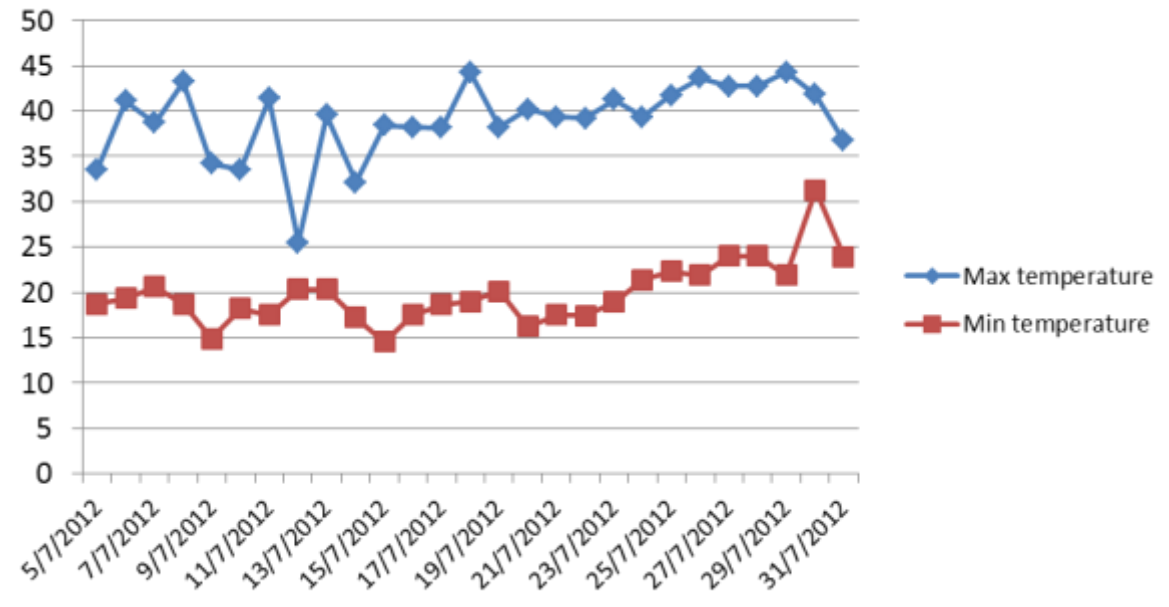
Degradability in soil



- ◆ Term = 28 days
- ◆ Volume of pot = 700 ml
- ◆ Composition = Sandy soil : gravel (2 : 1)
- ◆ 50 ml of water / day

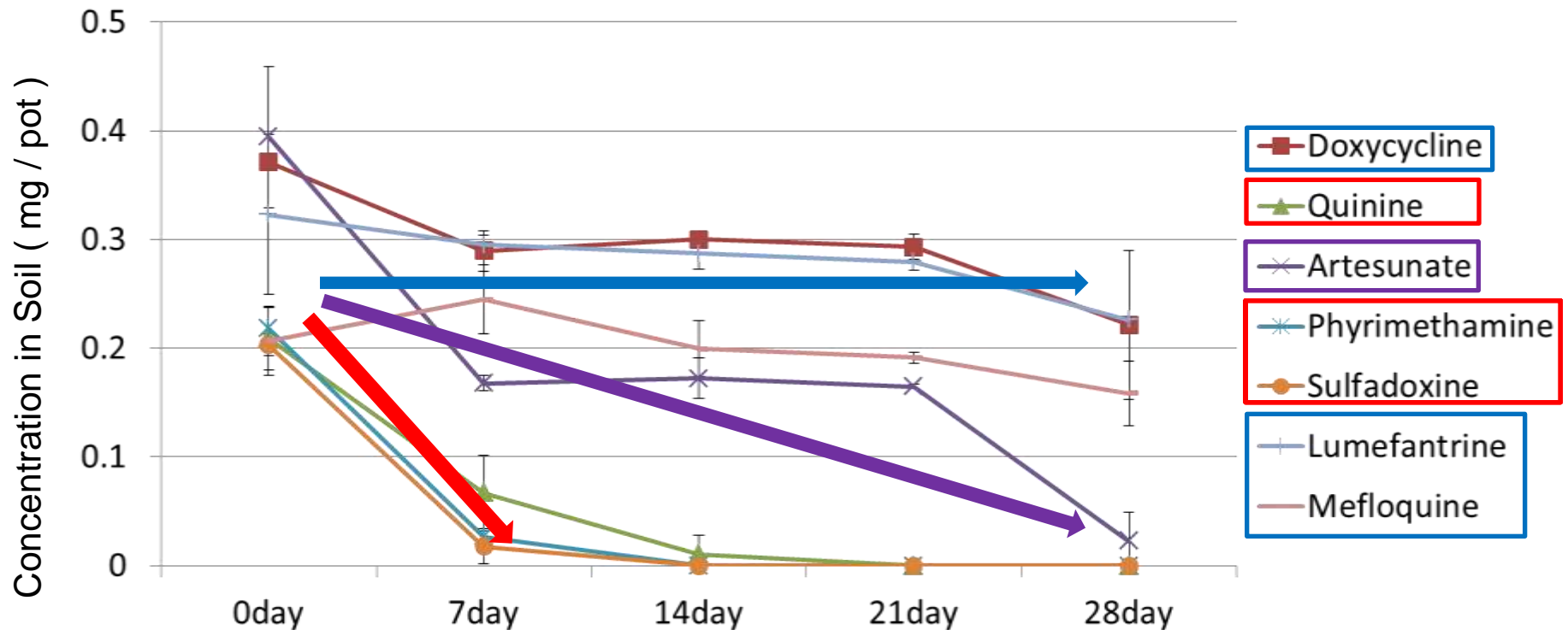
Exp1. Degradability in soil (without plant)

Add 0.30 mg each anti-malarial drug / pot



- ◆ pH = 6.7 ± 0.3
- ◆ EC = $100 \pm 30 \mu\text{S/cm}$
- ◆ Humidity Max = $64 \pm 13\%$, Min = $28 \pm 10\%$

Results ~Exp.1 Degradability in soil ~



Doxycycline, Lumefantrine and Mefloquine have **low degradability** in soil

Artesunate have **slow degradability** in soil

Quinine, Phyrimethamine and Sulfadoxine have **high degradability** in soil

Pot experiment (Komatsuna)



Exp2. Add all anti-malarial drugs *before seedling*

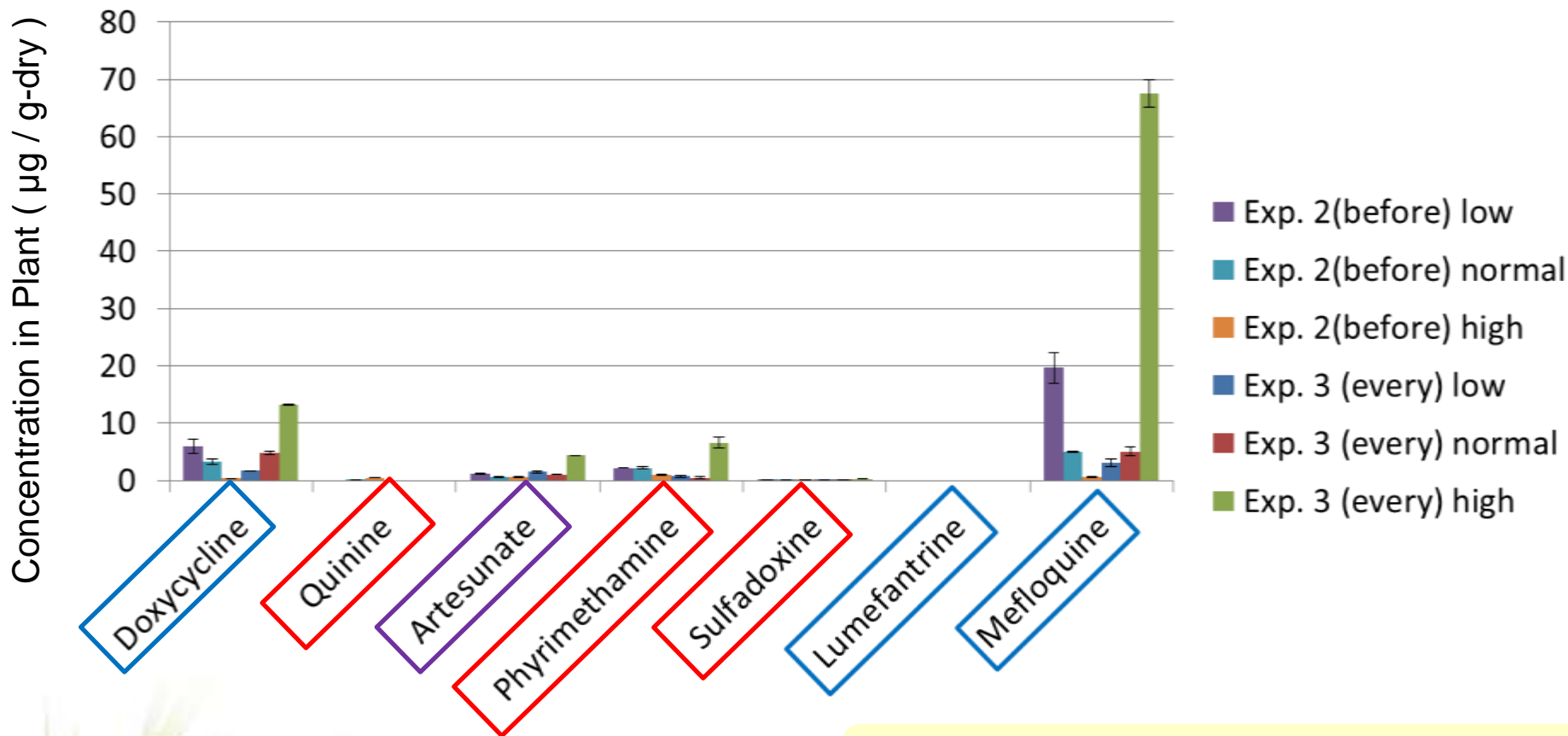
- ① normal concentration (0.30 mg/pot each)
- ② 10 times lower (0.03 mg/pot each)
- ③ 10 times higher (3.0 mg/pot each)

Exp3. Add anti-malarial drugs *every week*

- ① normal concentration ($0.075 \text{ mg/pot each}$)
- ② 10 times lower ($0.0075 \text{ mg/pot each}$)
- ③ 10 times higher (0.75 mg/pot each)

- ◆ Conditions were same as Exp1
- ◆ 7.03 ml of synthetic urine every week

Results ~Exp.2 & 3 (concentration in plant)





4 anti-malarial drugs have the potential to be taken up into plant

Some anti-malarial drugs was not taken up into plant

Why Lumefantrine was not taken up ?

Discussion

	MW	LogKow	pKa	Distribution
Carbamazepine	236	2.45	7	Plant
Melamine	126	-1.37	7	Plant
Diazinon	304			Plant
Florfenicol	358	-0.04	-	Plant
Levamisole	204	1.84	-	Plant
Tylosin	916			<u>Remained in soil</u>

MW and LogKow of Lumefantrine (529, 8.67) were most large in my study

Molecular weight and LogKow might be factors which affects the potential of pharmaceuticals to be taken up into plant

Conclusions



Investigation of method for determination of anti-malarial drugs in soil and plant

- ◆ Using mixture of methanol and KCl as extract solvent



Which anti-malarial drugs have potential to be taken up into plant ?

- ◆ Some anti-malarial drugs have potential to be taken up into plant
- ◆ Molecular weight and LogKow might be factors which affects the potential of anti-malarial drugs to be taken up into plant

Thank you
for your attention



Kiitos Suomi

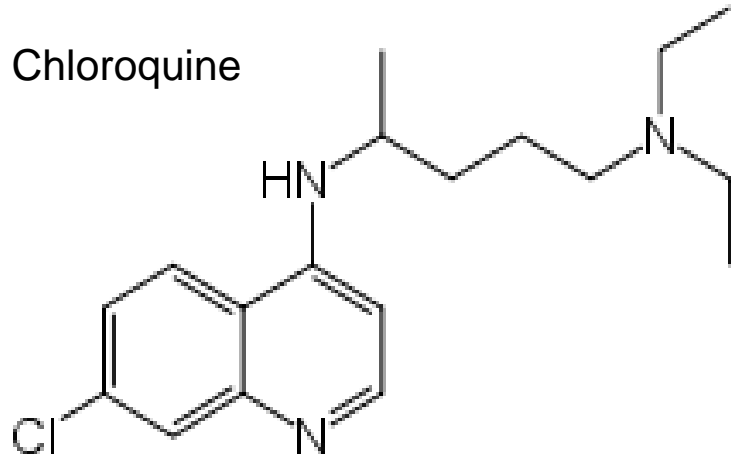
Conditions of LC / MS

LC condition	
Column	Sunfire (50 × 2.1mm, 3.0 μm)
Mobile phase	A: 10mM ammonium formate in pure water containing 0.3% formic acid
	B: acetonitrile
Column temperature	40°C

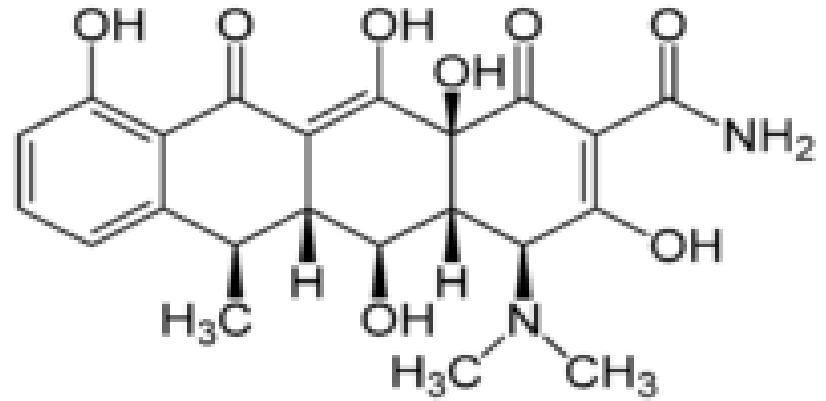
MS condition	
Ionization	ESI positive
Source temperature	140°C
Desolvation temperature	350°C
Gas flow rate	650L/hr
Corn desolvation	40L/hr

Anti-malarial drugs

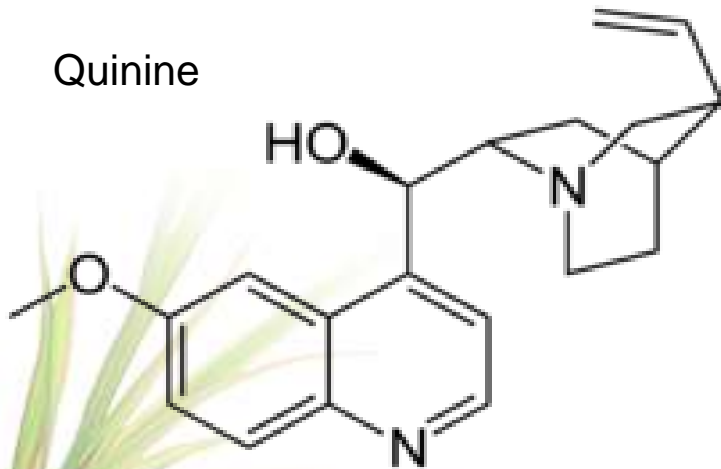
Chloroquine



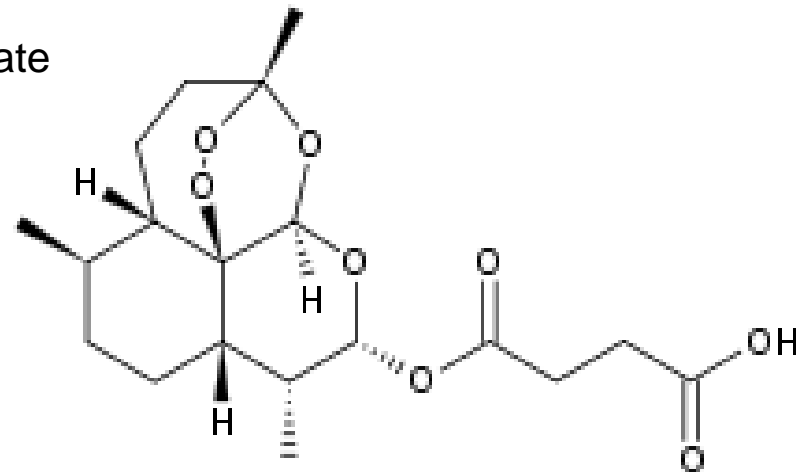
Doxycycline



Quinine

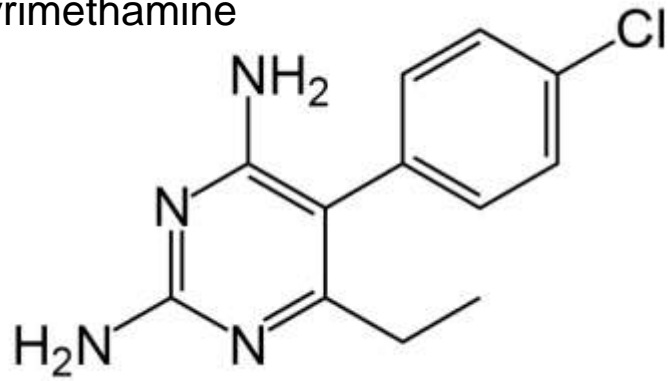


Artesunate

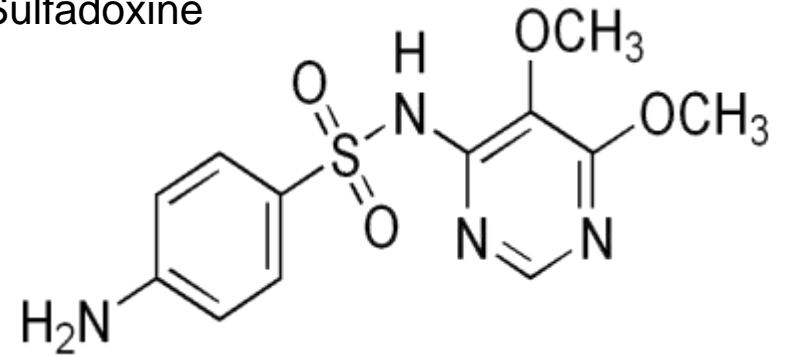


Anti-malarial drugs

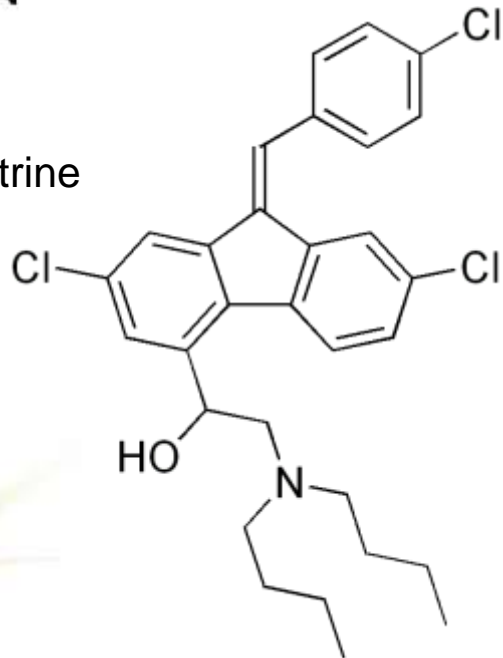
Pyrimethamine



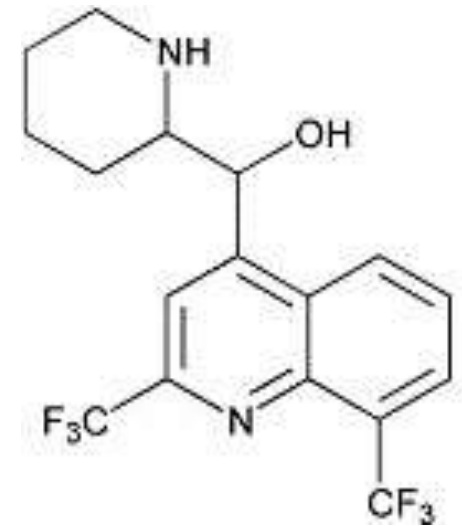
Sulfadoxine



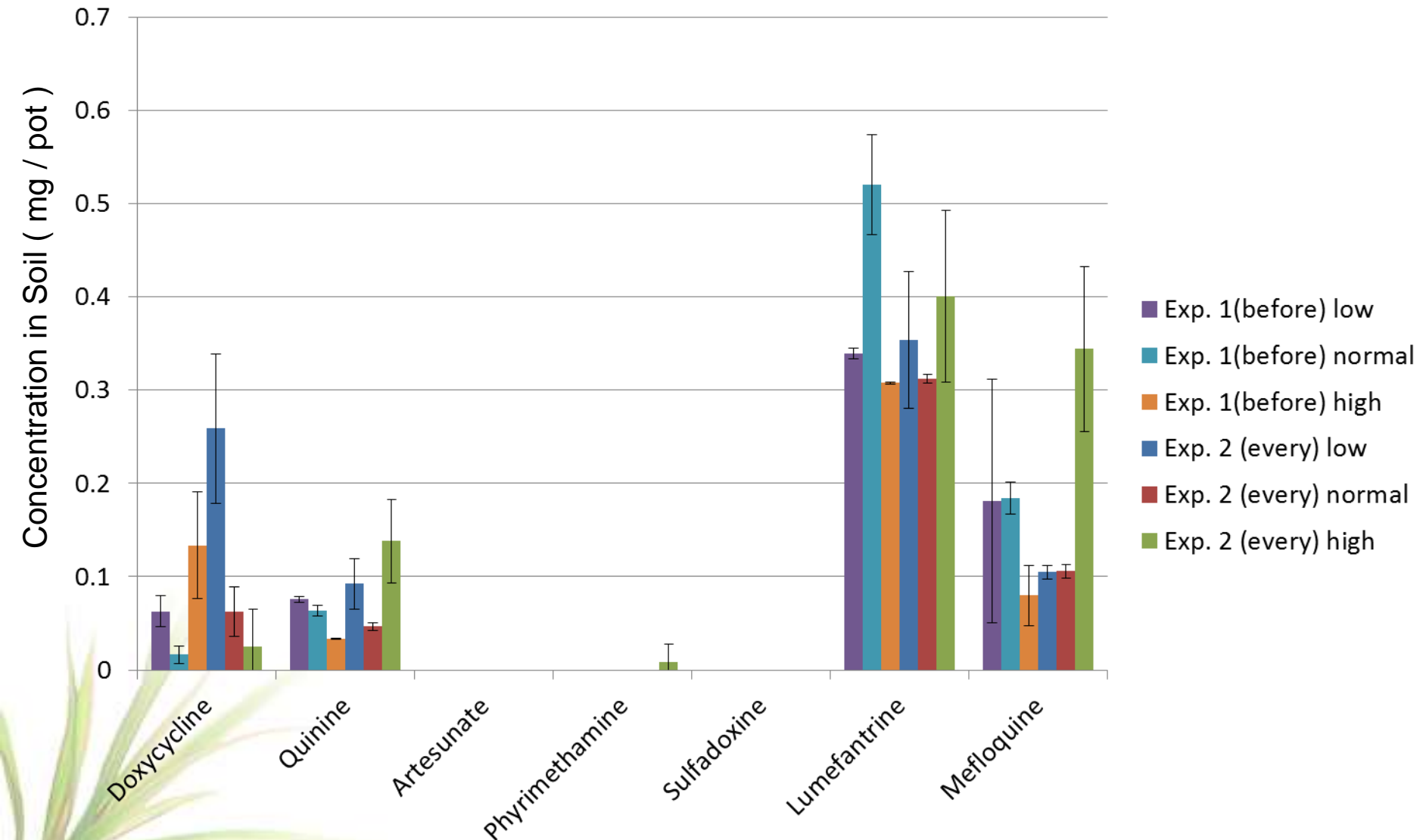
Lumefantrine



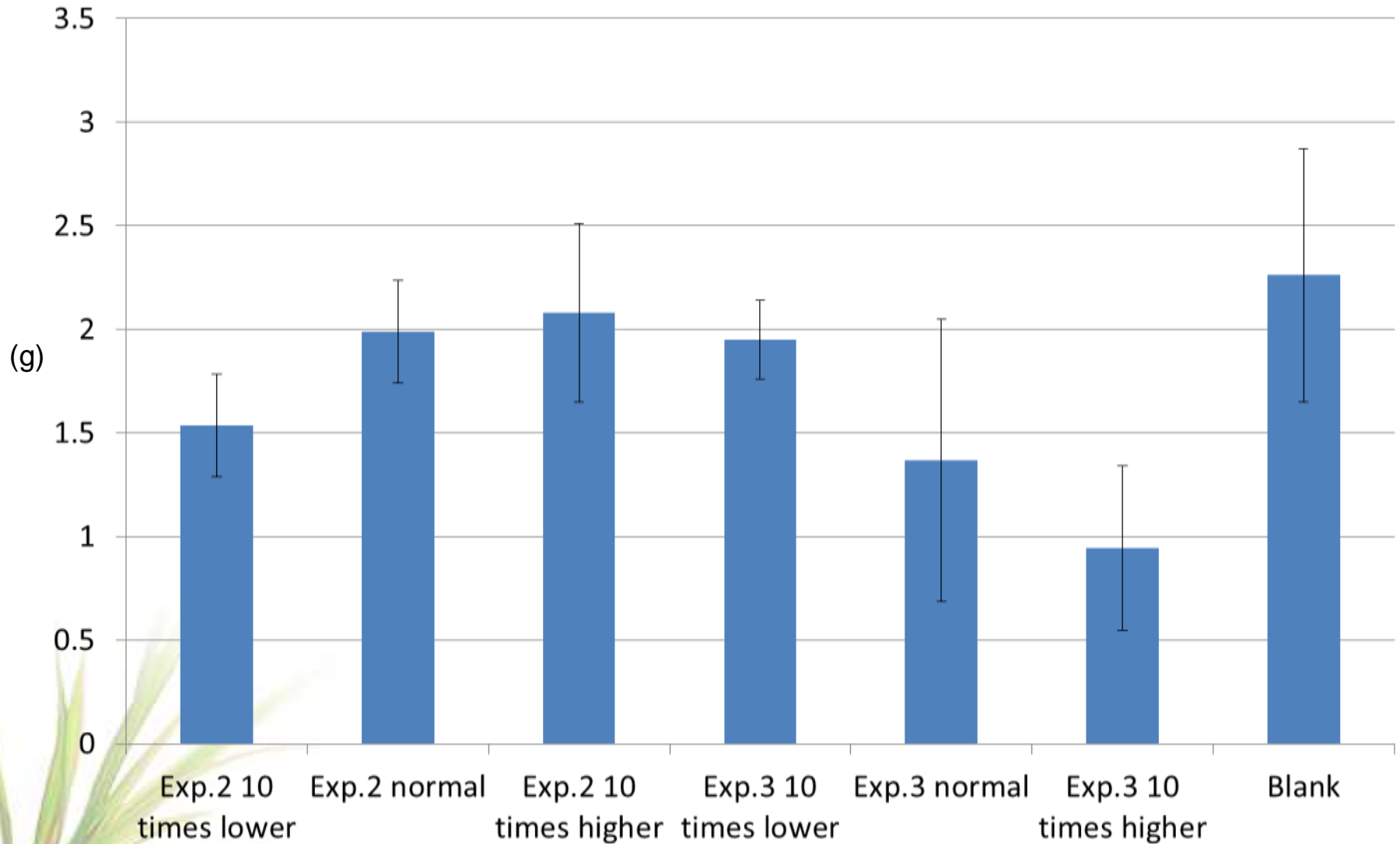
Mefloquine



Results ~Exp.1 and 2 (concentration in soil)



Dry weight of plant



Pot experiment (Komatsuna)



◆ Conditions were same as Exp1

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Plant required 1.4 kg/a - N

7.03 ml urine is needed

7.03 ml urine contain
0.30 mg drugs