"Key Scientific Drivers Behind Probiotic and Prebiotic Applications"
International Symposium of the International Scientific Association of Probiotics and Prebiotics
June 5-6, 2018, Furama Riverfront Hotel, Singapore

Detoxification of Environmental Chemicals With Probiotics

Gregor REID
Canada

Organised by:
INTERNATIONAL SCIENTIFIC ASSOCIATION OF PROBIOTICS AND PREBIOTICS™

In affiliation with:
Singapore Society for Microbiology and Biotechnology

Meeting Sponsors:
Platinum Yakult
Silver P&G

Conference Secretariat & Housing Bureau:
ace:daytons direct

Detoxification of environmental chemicals with probiotics.

Gregor Reid and Brendan Daisley

Lawson Health Research Institute
University of Western Ontario, London, Canada

gregor@uwo.ca
Tel: 519-646-6100 x65256

LAWSON HEALTH RESEARCH INSTITUTE

Western UNIVERSITY · CANADA

Our food and water are contaminated.

•No surprise since we're drinking what the dinosaurs drunk!
•In developing countries, **70 percent of industrial wastes** are dumped untreated into waters, polluting the usable water supply.
•40% of the world's rivers and lakes are too contaminated to drink from or wash in.
•On average, **99 million pounds** (45 million kilograms) of fertilizers and chemicals are used each year.
•15% of Canadian women of reproductive age have **mercury** levels above the levels where neurodevelopmental abnormalities may occur in children

Our air and water

SCIENTIFIC AMERICAN™

Subscribe | News & Features | Topics | Blogs | Multimedia | Education | Citiz

Health :: Forum :: October 11, 2011 :: 12 Comments :: Email :: Print

Toxins All around Us

Exposure to the chemicals in everyday objects poses a hidden health threat

By Patricia Hunt

See Inside

Chemistry for a New Era

The International Year of Chemistry commemorates the achievements that have made life better. Breakthroughs promise a greener and more productive future »

October 10, 2011

Susan starts her day by jogging to the edge of town, cutting back through a cornfield for an herbal tea at the downtown Starbucks and heading home for a shower. It sounds like a healthy morning routine, but Susan is in fact exposing herself to a rogue's gallery of chemicals: pesticides and herbicides on the corn, plasticizers in her tea cup, and the wide

"An increasing number of clinicians and scientists are becoming convinced that these chemical exposures contribute to obesity endometriosis, diabetes, autism, allergies, cancer and other diseases, and fetuses are particularly vulnerable"

- Rauch and Lanphear (2012) reported that **many disabilities of childhood** have their roots in the environment--from toxins in air, water, and soil, to the stressors of poverty, to marketing practices that encourage unhealthy choices or discourage healthy ones.

Pesticides

- We use protective equipment while spraying but then consume that same food
- It's not surprising that pesticide exposure has been linked to many diseases:

Elevated Serum Pesticide Levels and Risk for Alzheimer Disease

Jason R. Richardson, PhD[1,2]; Ananya Roy, ScD[2]; Stuart L. Shalat, ScD[1,2]; Richard T. von Stein, PhD[2]; Muhammad M. Hossain, PhD[1,2]; Brian Buckley, PhD[2]; Marla Gearing, PhD[4]; Allan I. Levey, MD, PhD[3]; Dwight C. German, PhD[5]

JAMA Neurol. 2014;71(3):284-290. doi:10.1001/jamaneurol.2013.6030.

Pesticide use and colorectal cancer risk in the agricultural health study

Won Jin Lee, Dale P. Sandler, Aaron Blair, Claudine Samanic, Amanda J. Cross, Michael C.R. Alavanja

Parkinson's disease and brain levels of organochlorine pesticides

Lora Fleming MD, MPH, John B. Mann MS, Judy Bean PhD, Thomas Briggle PhD, Dr. Juan R. Sanchez-Ramos MD, PhD

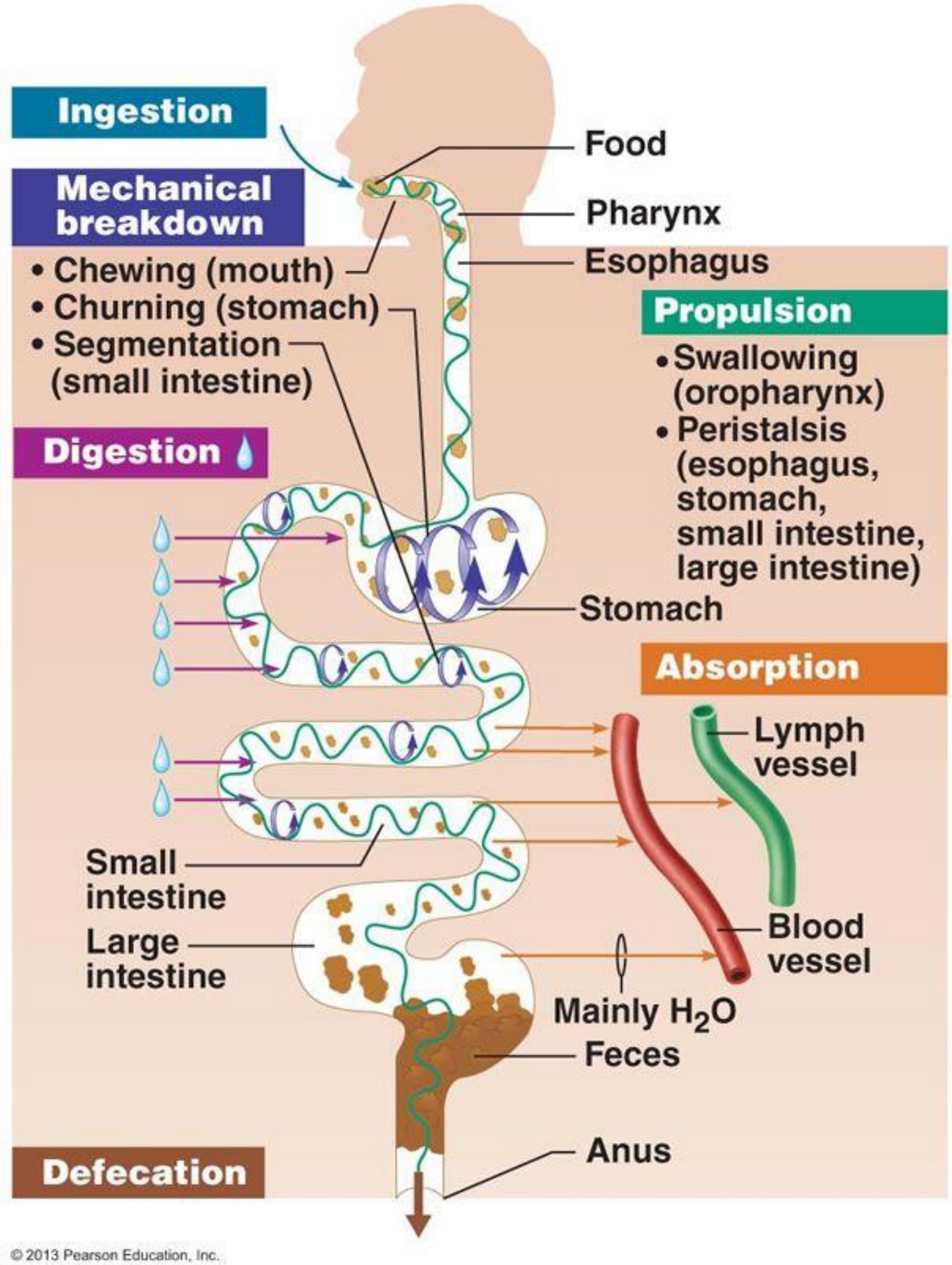
?

Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives

Sara Mostafalou, Mohammad Abdollahi

Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

?



https://www.google.com/search?q=image+of+digestion&client=firefox-b&tbm=isch&source=iu&ictx=1&fir=RXo_fPJyhN4NrM%253A%252CbE3Z8NJgi4DtXM%252C_&usg=__-OVk3-kAhWntsGLmc6Ios0gcz8Q%3D&sa=X&ved=0ahUKEwjNt4ry6orbAhXK50MKHS2gAroQ9QEIPTAK#imgrc=RXo_fPJyhN4NrM:

So, if we can't stop the pollution, how do we stop the toxins affecting us?

1. Prevent adsorption of the toxins
2. Degrade the toxins
3. Counter the damage they cause

www.bbc.com/news/av/world-africa-42128779/scientists-warn-lake-victoria-is-dying

lake victoria is dying

Dynamics and associa...

Scientists warn Lake Victoria is dying

Scientists are warning that Lake Victoria, Africa's largest freshwater lake, is under threat of dying.

They blame overfishing and pollution for severely damaged fish stocks.

Amina Yuguda, the **BBC World News Komla Dumor Award winner**, reports from Uganda.

27 Nov 2017 | Africa

Paper 12

Beneficial Microbes, 2018 online

ARTICLE IN PRESS

Wageningen Academic Publishers

Expanding the reach of probiotics through social enterprises

G. Reid[1*], R. Kort[2,3,4], S. Alvarez[5], R. Bourdet-Sicard[6], V. Benoit[7], M. Cunningham[8], D.M. Saulnier[9], J.E.T. van Hylckama Vlieg[10], H. Verstraelen[11] and W. Sybesma[2]

[1]Canadian Research and Development Centre for Probiotics, Microbiology & Immunology, and Surgery, University of Western Ontario, Room F3-106, P.O. Box 5777, STN B , London, N6A 4V2 Ontario, Canada; [2]Yoba for Life foundation, Hunzestraat 133-A, 1079 WB Amsterdam, the Netherlands; [3]TNO Microbiology and Systems Biology, P.O. Box 360, 3700 AJ Zeist, the Netherlands; [4]VU University Amsterdam; Micropia, Natura Artis Magistra, Plantage Kerklaan 38-40, 1018 CZ Amsterdam, the Netherlands; [5]Reference Centre for Lactobacilli (CERELA-CONICET), Chacabuco 145, Tucuman 4000, Argentina; [6]Danone Access, Africa & India, Danone Nutricia Research, Avenue de la Vauve, 91767 Palaiseau, France; [7]General Mills, Nutrition and Technology Solutions, 9000 Plymouth Avenue N, Minneapolis, MN 55427, USA; [8]Research and Development, Metagenics (Aust) Pty Ltd., P.O. Box 675, Virginia BC, Queensland 4014, Australia; [9]Novozymes A/S, Hillerødgade 42, 2200 Frederiksberg, Denmark; [10]Chr Hansen AS, Bøge Alle 10, 2970 Hoersholm, Denmark; [11]Vulvovaginal Disease Clinic, Dept. of Obstetrics & Gynaecology, Ghent University Hospital 0P4, Corneel Heymanslaan 10, 9000 Gent, Belgium; gregor@uwo.ca

Study Design: Pregnant Mothers

- 61 Mothers enrolled in 2nd trimester
- 26 received probiotic yogurt, 30 no intervention
- Monthly sampling of oral, vaginal and gut microbiota until birth
- Sampling of breast milk and infant oral and gut after birth for one month

Study Design: Children

- In addition to previous cohort of mothers, also tested in 44 school-aged children
- Randomized to consume either *L. rhamnosus* GR-1 yogurt or milk (control) over 25-days

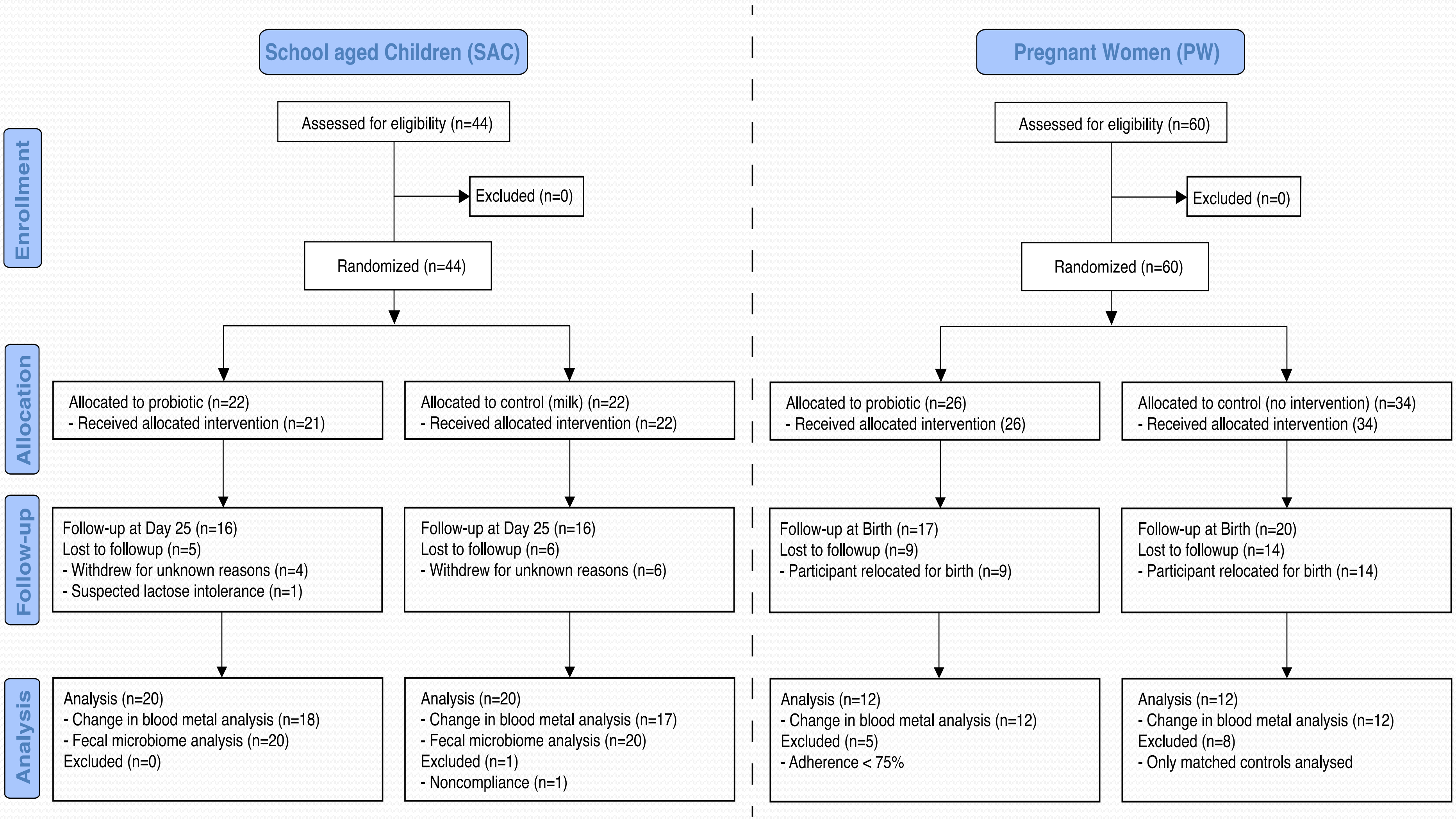


Fig 1 CONSORT flow diagram for study cohorts.

Baseline toxic metal levels

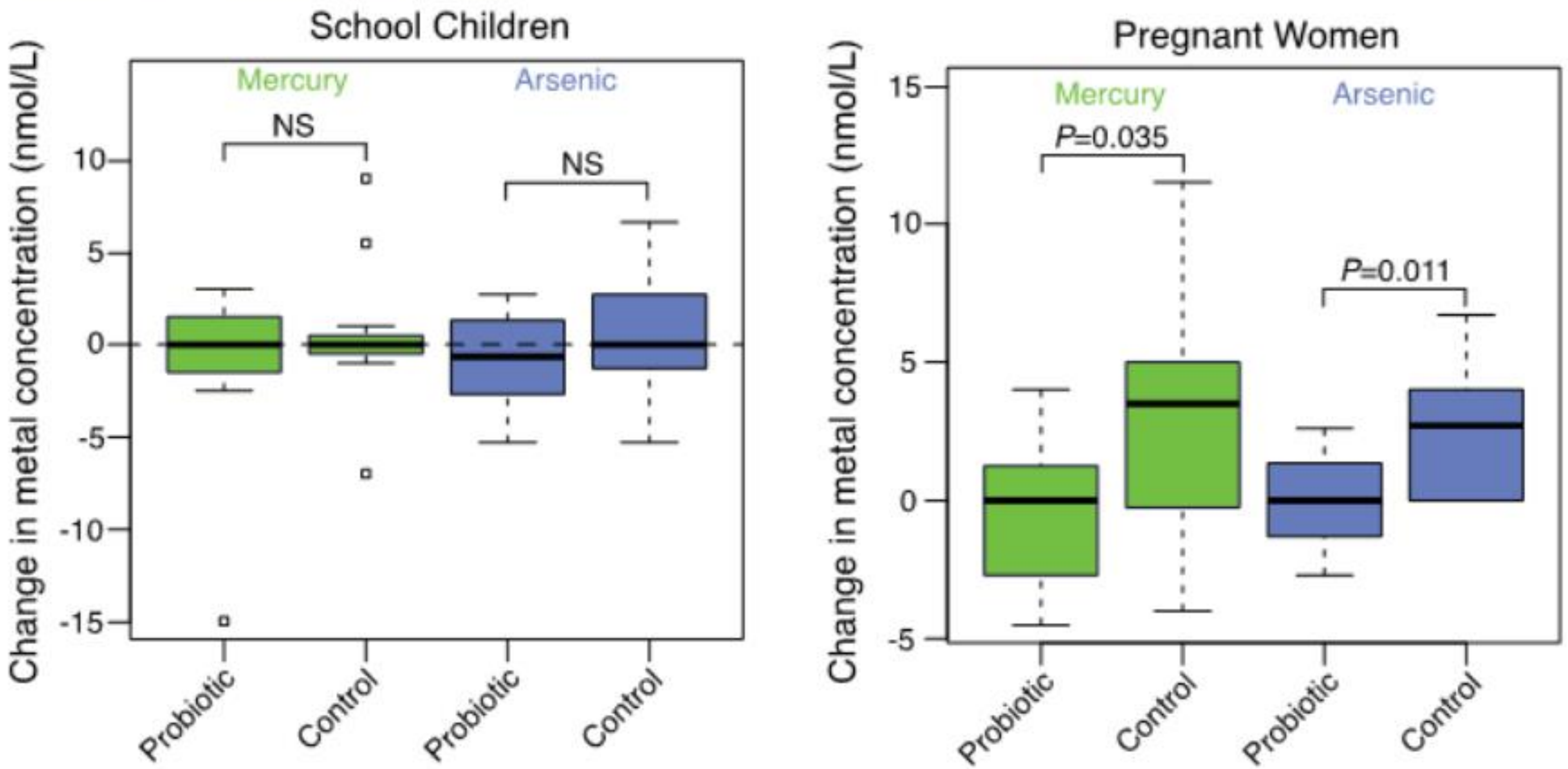
TABLE 3 Blood metal levels at the time of recruitment and comparisons to levels found in a developed country

Study group and heavy metal	Metal level in test group		Metal level in controls		Fold difference
	Avg ± SD	Range	Canadian avg[a]	Reference range[b]	
SAC					
Pb (μg/liter)	47.1 ± 16.2	22.5–91.3	9.0	0.0–17.7	5.2
Hg (nmol/liter)	9.5 ± 5.3	3.0–37.4	1.4	0.0–5.5	6.8
As (nmol/liter)	6.5 ± 2.1	2.7–10.8	7.8	0.0–21.4	−1.2
Cd (nmol/liter)	1.2 ± 0.7	0.9–4.4	0.89	0.0–4.6	1.3
PW					
Pb (μg/liter)	22.6 ± 9.6	7.3–40.5	8.9	0.0–45.0	2.5
Hg (nmol/liter)	8.8 ± 3.1	4.0–16.0	3.5	0.0–18.0	2.5
As (nmol/liter)	3.0 ± 1.6	1.3–6.7	11.7	0.0–21.4	−3.9
Cd (nmol/liter)	1.1 ± 0.6	0.0–2.7	3.2	0.0–8.9	−2.9

[a] Canadian averages are geometric means for males and females ages 6 to 11 years (SAC) and of females ages 20 to 39 years (PW) and are based on the Canadian Health Measures Survey (2007-2009 [13]).

[b] Reference ranges were provided by the Trace Elements Laboratory, London Laboratory Services Group.

Effect of probiotic on metal levels



Conclusion: no statistical effect in children (1 month intervention) but effect in pregnant women (~4 months intervention).

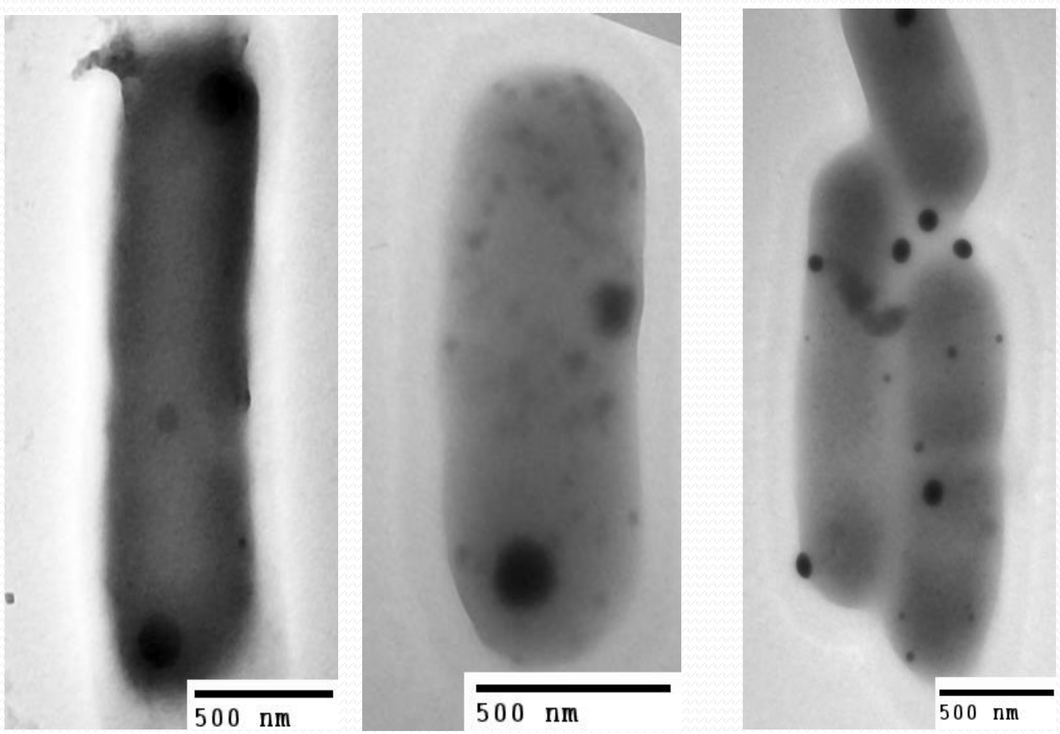
LAWSON HEALTH RESEARCH INSTITUTE

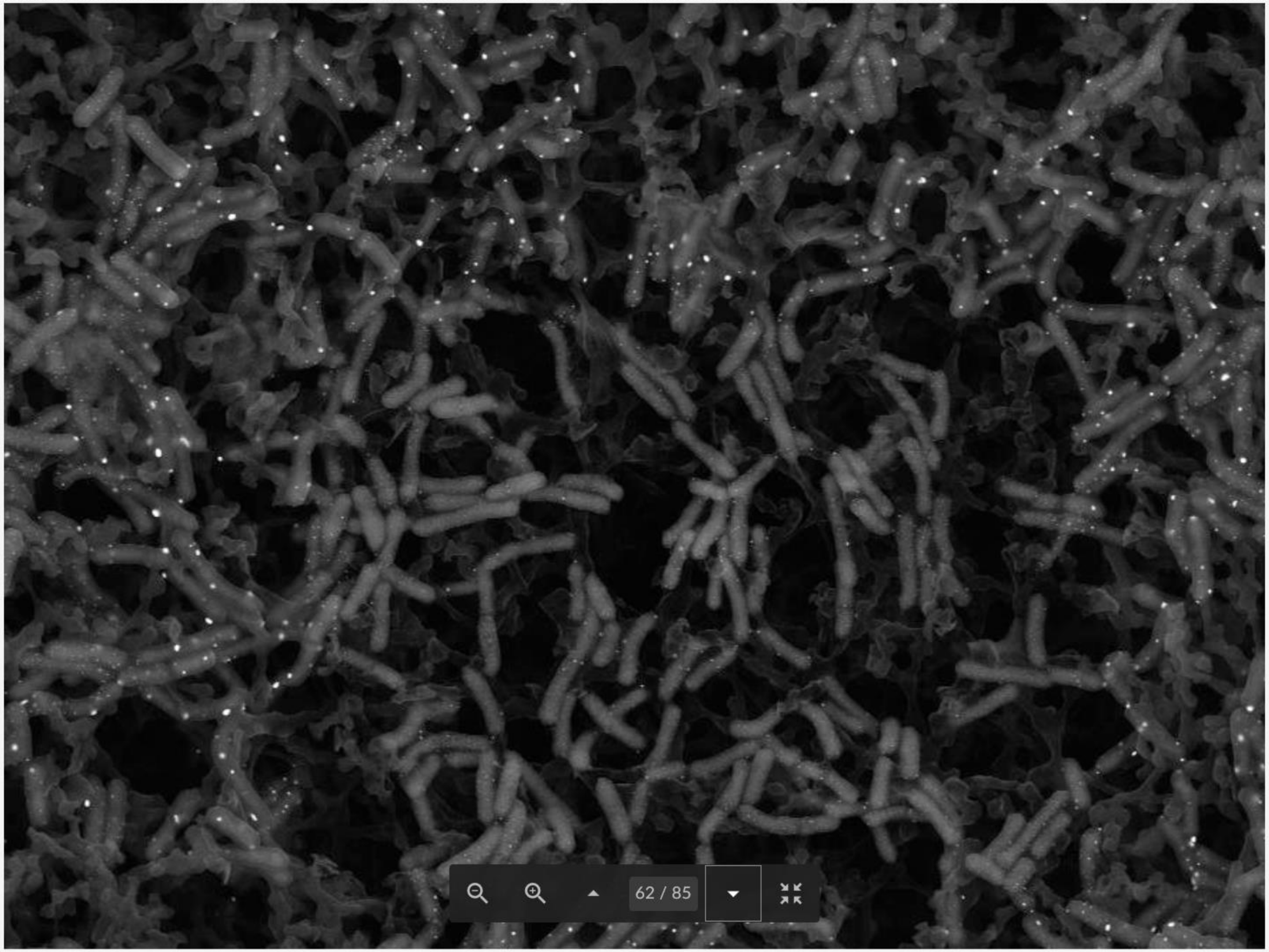
Randomized Open-Label Pilot Study of the Influence of Probiotics and the Gut Microbiome on Toxic Metal Levels in Tanzanian Pregnant Women and School Children

Jordan E. Bisanz,[a,b] Megan K. Enos,[a,b] Joseph R. Mwanga,[f] John Changalucha,[f] Jeremy P. Burton,[a,b,c,d] Gregory B. Gloor,[e] Gregor Reid[a,b,c]

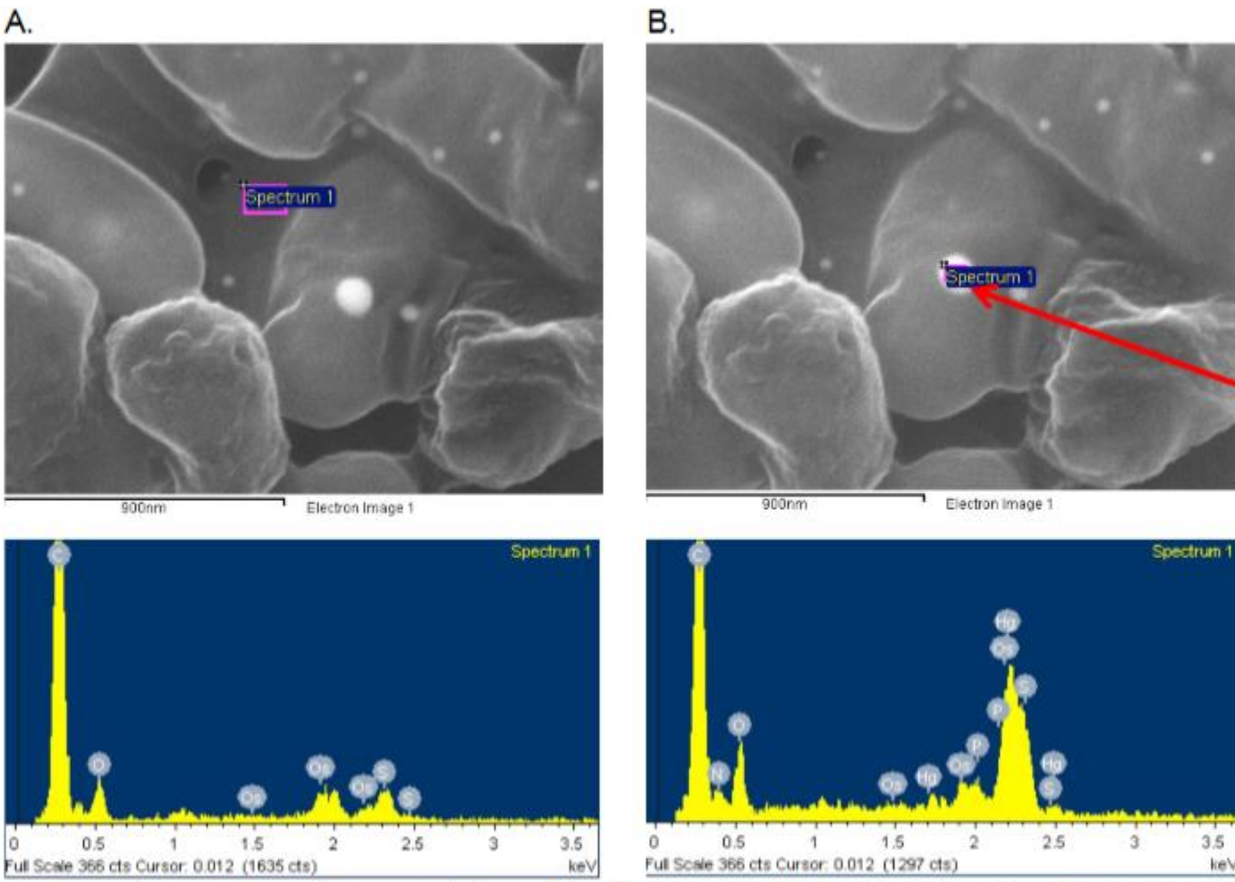
Result:
Daily intake of Lactobacillus rhamnosus GR-1 supplemented probiotic yogurt, for over three months in pregnant women resulted in **36% less mercury and 75% less arsenic** adsorbed.

A. Control B. Lead C. Mercury




62 / 85

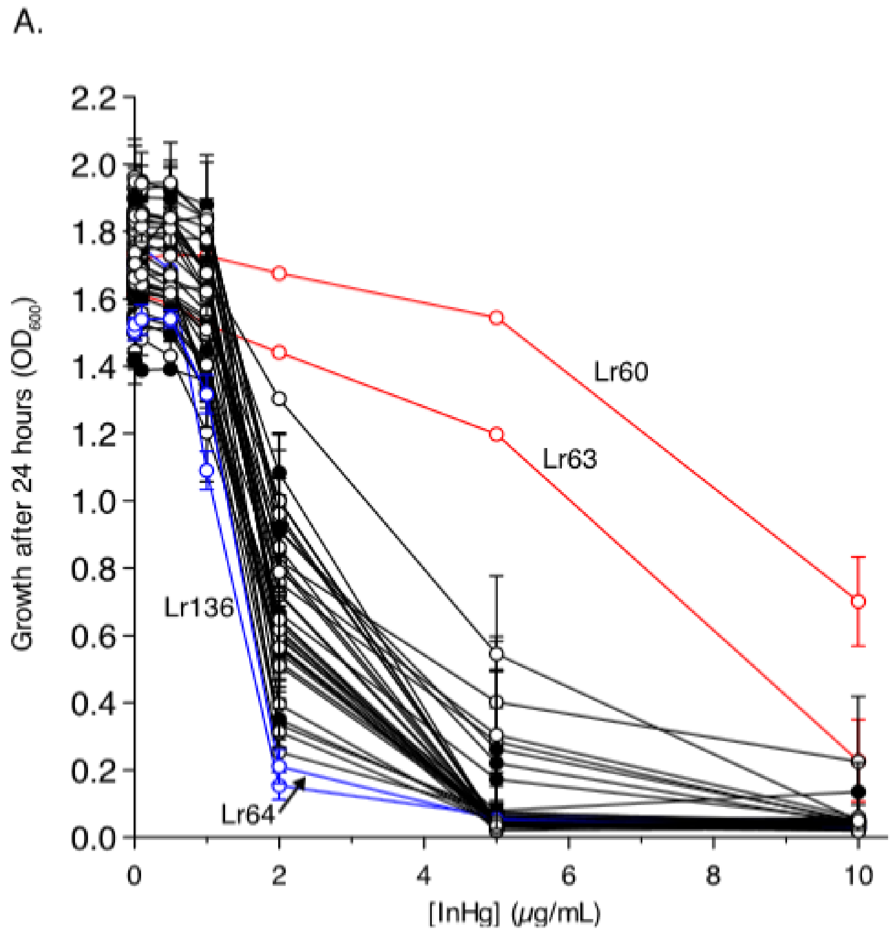
Metal sequestration by lactobacilli



Element	Weight%	Atomic%
C K	39.67	70.99
N K	7.51	11.52
O K	8.51	11.44
P K	1.23	0.85
S K	0.97	0.65
Os M	5.5	0.62
Hg M	36.62	3.92
Totals	100	

EDX analysis confirms that precipitates are predominantly toxic metals by elemental composition.

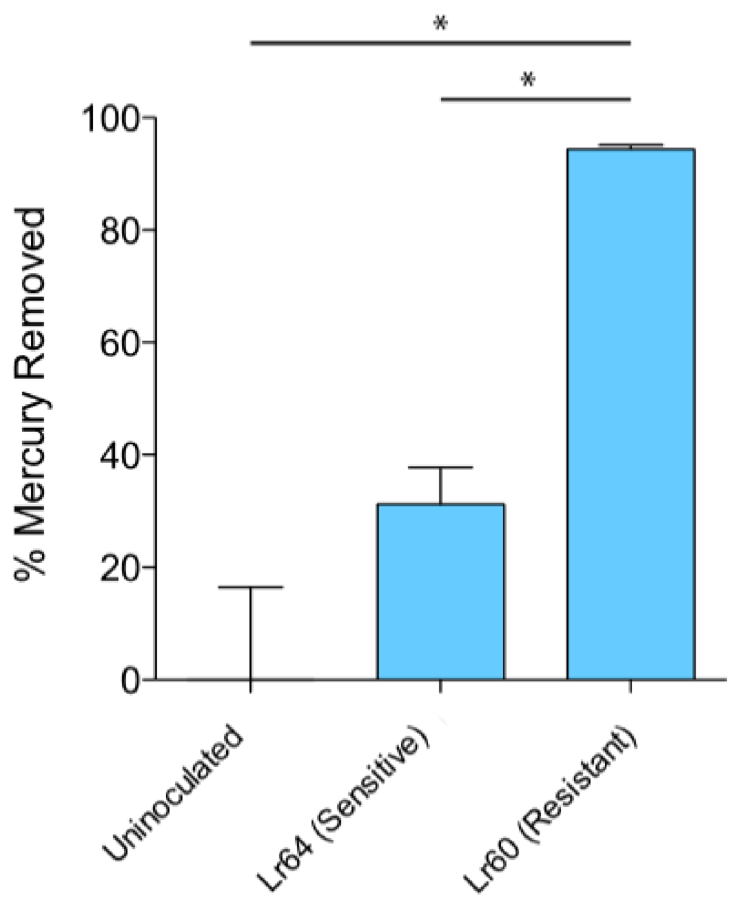
Hg²⁺ resistance as proxy for *merA*



80+ strains growth monitored after 24h in a gradient of Hg^{2+}

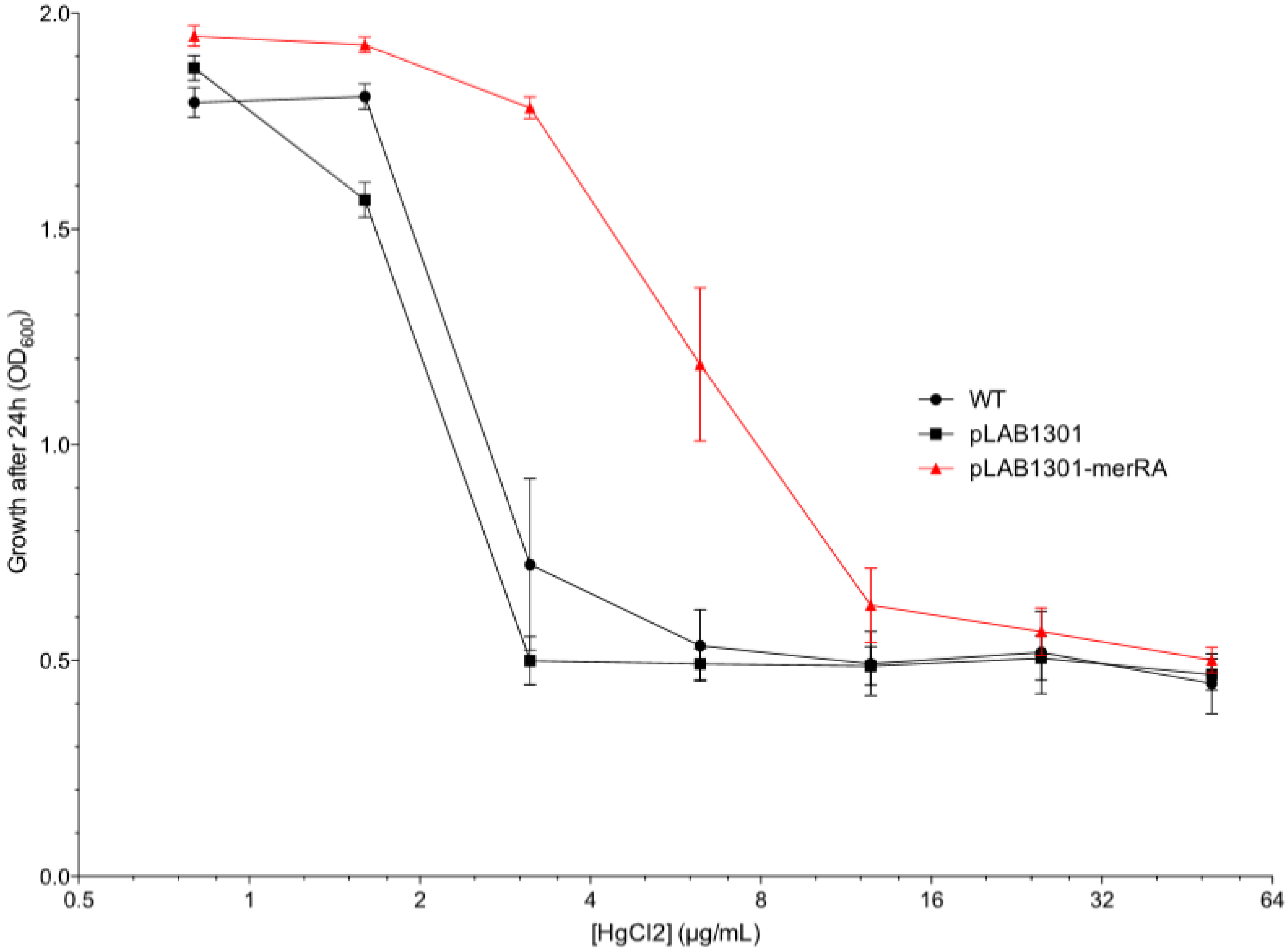
LAWSON
HEALTH RESEARCH INSTITUTE

Effect of mercury resistance on remediation



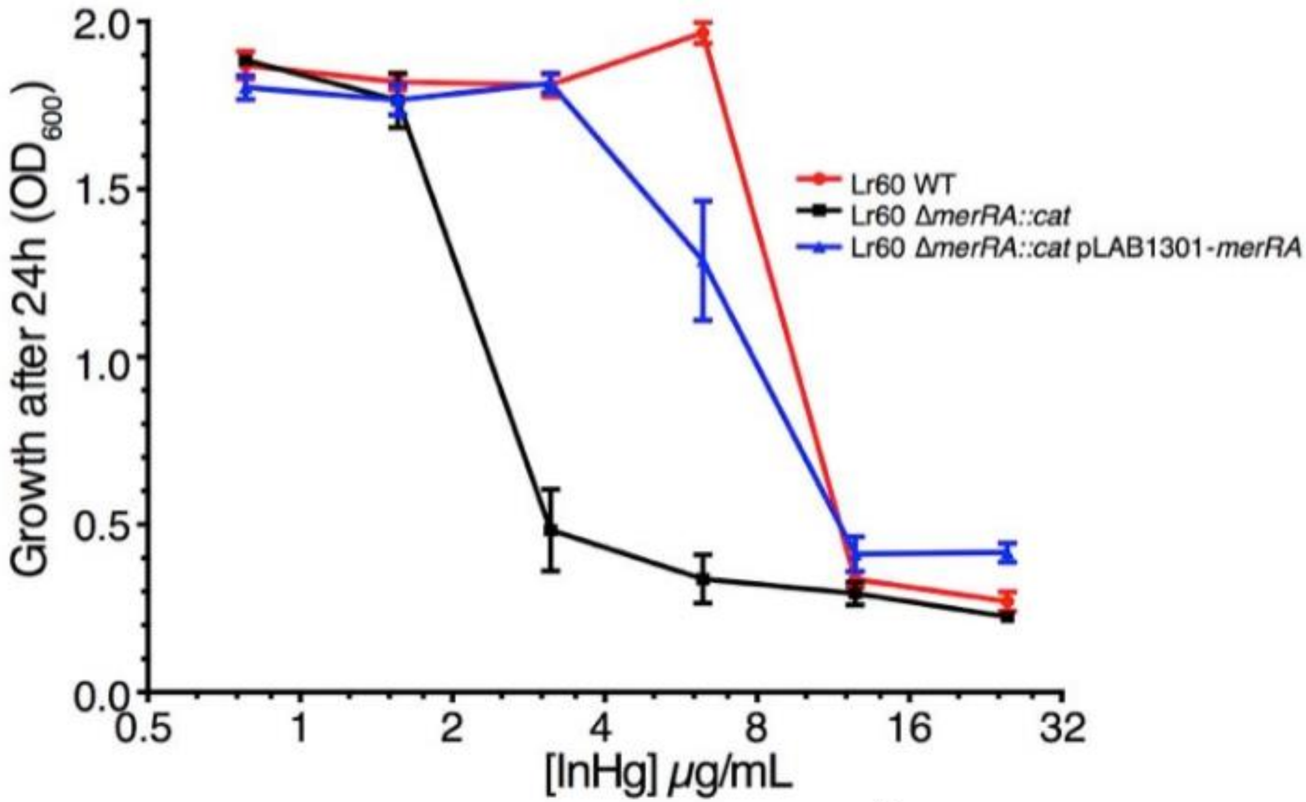
1 ppm starting inoculum

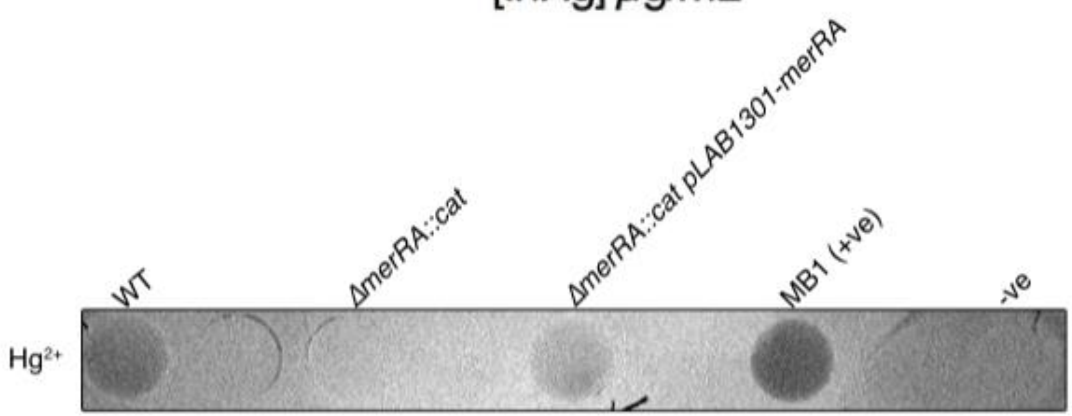
LAWSON
HEALTH RESEARCH INSTITUTE



Expression of *merRA* from *L. rhamnosus* Lr60 *in L. rhamnosus* GR-1 increases the minimum inhibitory concentration 4-fold.

LAWSON
HEALTH RESEARCH INSTITUTE

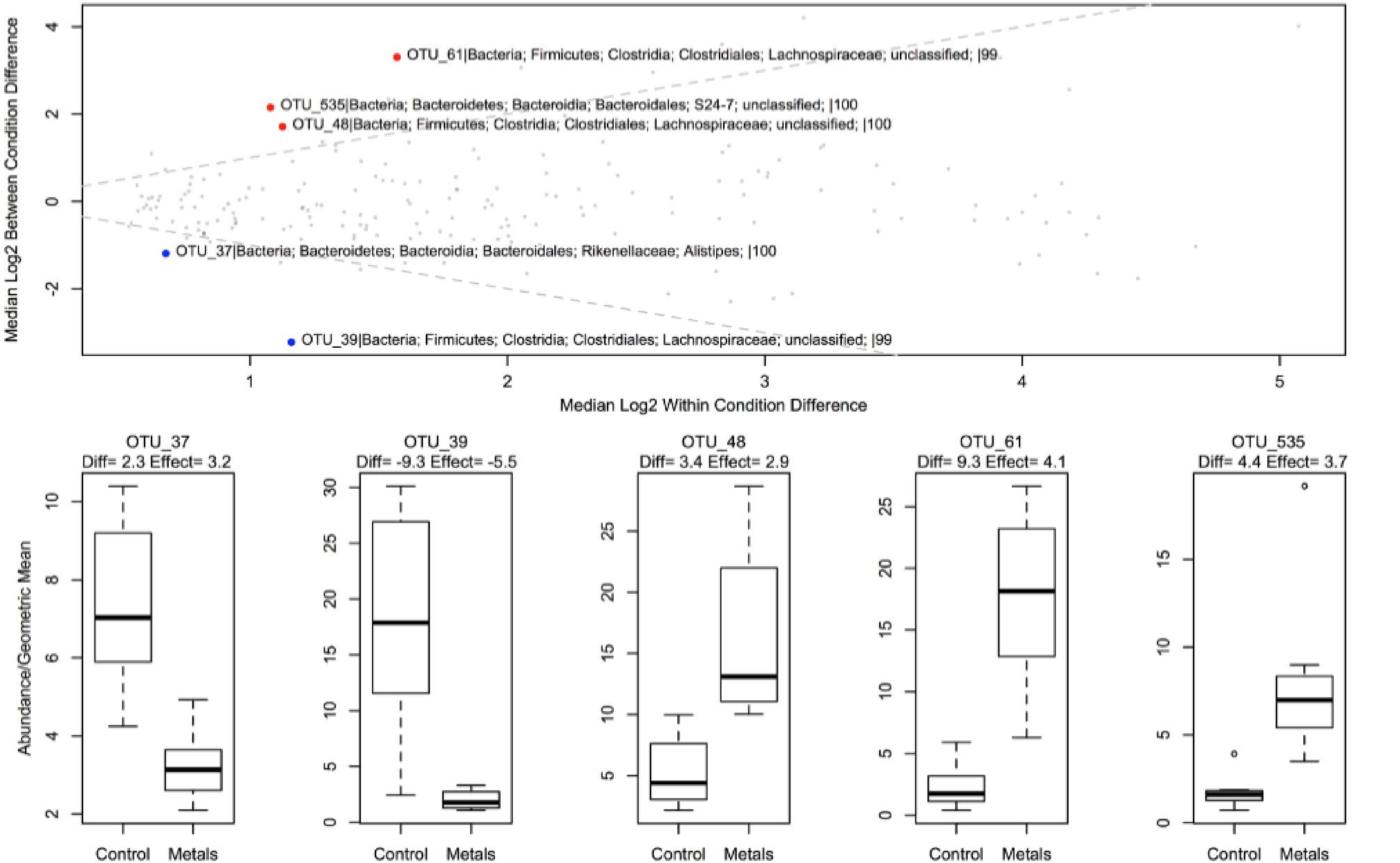


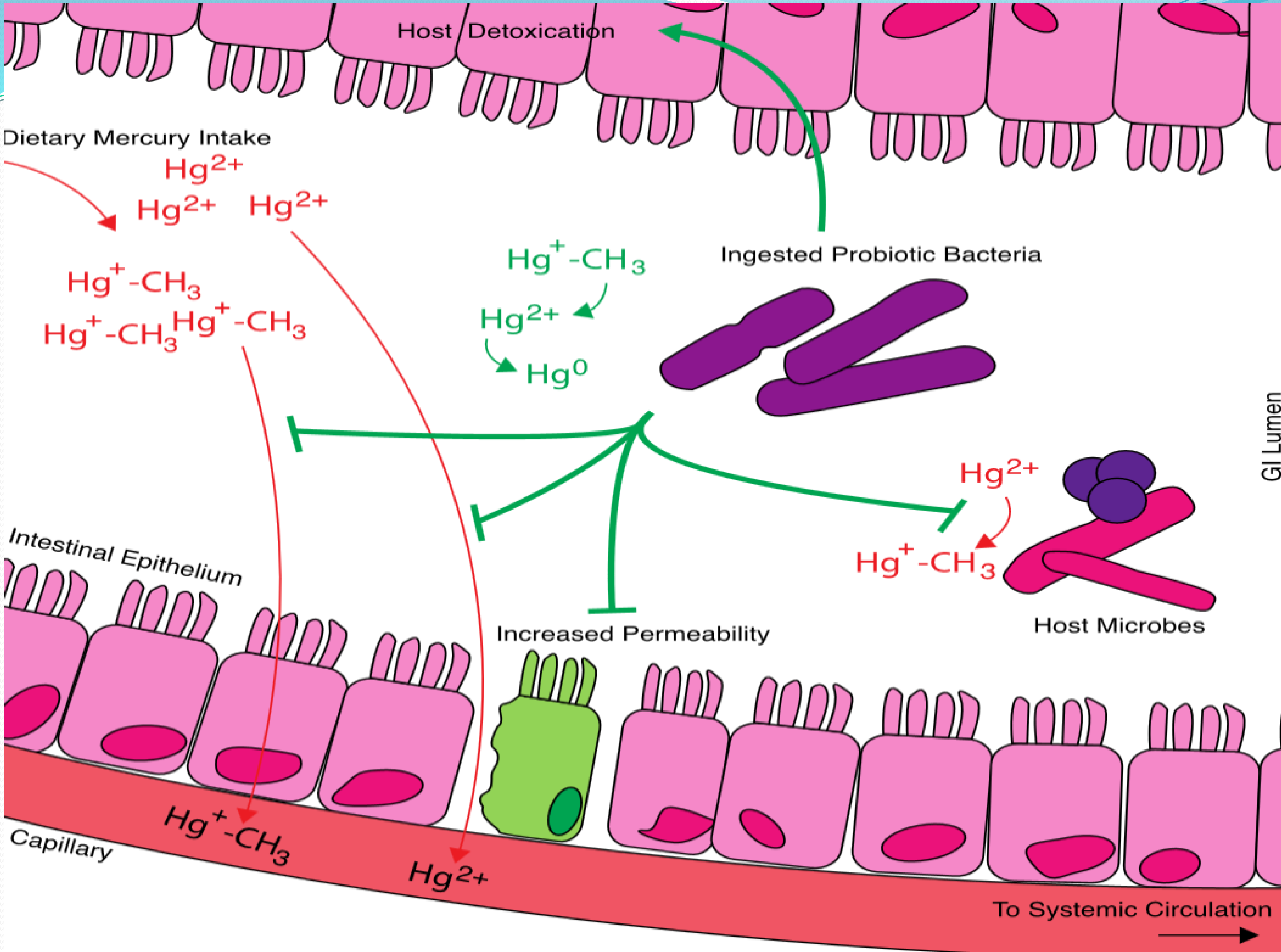


Knockout of *merRA* in *L. rhamnosus* Lr60 causes loss of mercury resistance and volatilization which is recovered by complementation

How does the microbiota respond to toxic metals?

Mouse studies





Host Detoxication
Dietary Mercury Intake
Hg2+
Hg2+ Hg2+
Hg+-CH3
Hg+-CH3 Hg+-CH3
Hg+-CH3
Hg2+
Hg0
Ingested Probiotic Bacteria
GI Lumen
Hg2+
Hg+-CH3
Host Microbes
Intestinal Epithelium
Increased Permeability
Capillary
Hg+-CH3
Hg2+
To Systemic Circulation

Aflatoxins are one of the most highly toxic secondary metabolites posing a serious threat to human and animal health by hepatotoxicity, teratogenicity, and immunotoxicity.

They are produced by fungal species such as *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*, which usually infect cereal crops including wheat, walnut, corn, cotton, peanuts and tree nuts.

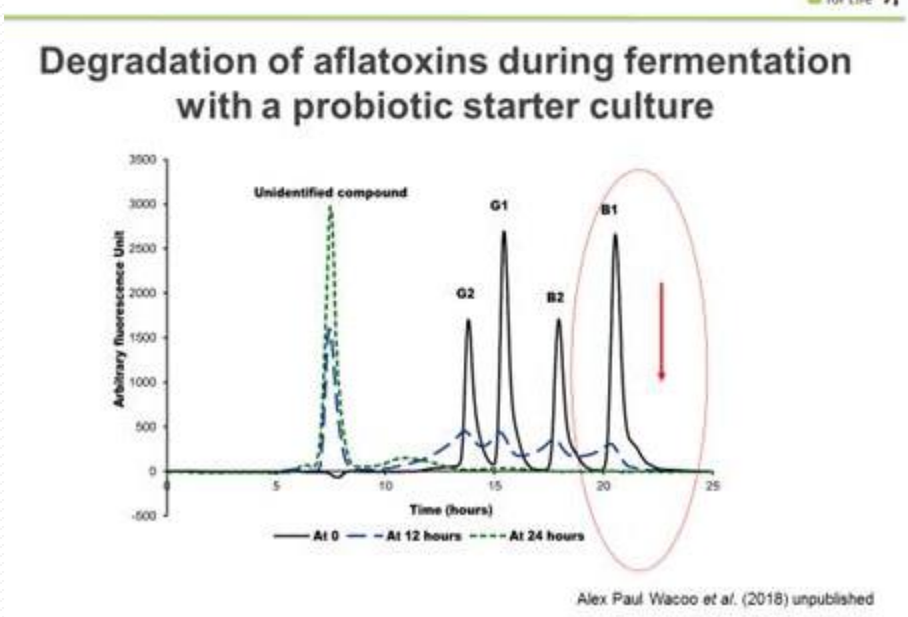
The major aflatoxins are B1, B2, G1, and G2, which can poison the body through respiratory, mucous or cutaneous routes, resulting in overactivation of the inflammatory response. *Kumar et al. 2016*

Children aged 1-3 years require a daily protein intake of 13 g (WHO, 2013) and an energy intake of 902 – 1046 Kcal (FAO, 2004).

Daily consumption of up to 1000 ml of traditional cereal porridge helps meet up to 44% and 33% of the daily protein and energy requirements, respectively.

But, about 50% of traditionally produced commercial millet in Kampala does not meet safety requirements for pathogens and highly toxic aflatoxins that stunt growth and cause cancer.

Fermentation by Yoba cultures in community kitchens in Uganda acidifies the
, and **breaks down the major**



Neonicotinoids and CCD

- One of the factors involved in Colony Collapse Disorder

Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees

Gennaro Di Prisco[a], Valeria Cavaliere[b], Desiderato Annoscia[c], Paola Varricchio[a], Emilio Caprio[a], Francesco Nazzi[c], Giuseppe Gargiulo[b], and Francesco Pennacchio[a,1]

Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide

Dara A. Stanley, Karen E. Smith & Nigel E. Raine

Four Common Pesticides, Their Mixtures and a Formulation Solvent in the Hive Environment Have High Oral Toxicity to Honey Bee Larvae

Wanyi Zhu, Daniel R. Schmehl, Christopher A. Mullin, James L. Frazier

- Immunosuppression
- Learning deficits
- Overt neurotoxicity

S

Imidacloprid
Thiamethoxam
Clothianidin

Experimental Design

- We test the effects of the highly controversial neonicotinoid insecticide, <u>**imidacloprid**</u>

70% of dead bees in Ontario test positive for neonicotinoid residues (Government of Ontario, 2013).

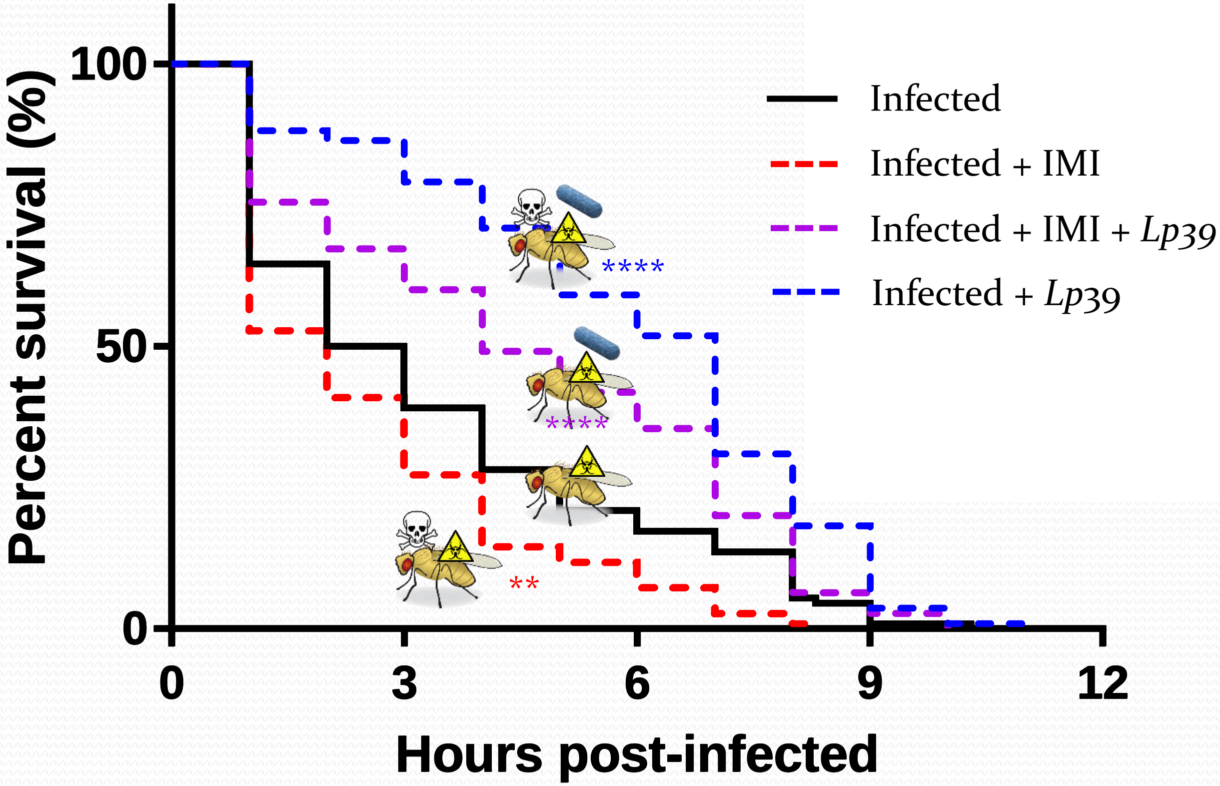
- Here, we use *Drosophila melanogaster* as a high-throughput model organism to better characterize how imidacloprid affect insect physiology

Drosophila melanogaster as a model for the honey bee

Both are similarly affected by imidacloprid
Both have a lactobacilli-dominated microbiota
Both share a highly conserved innate immune system

- **Hypothesis:** Probiotic lactobacilli can reduce imidacloprid-induced susceptibility to infection

- Supplementation with *Lactobacillus plantarum* 39 is able to rescue imidacloprid-induced susceptibility to *Serratia marcescens* infection.

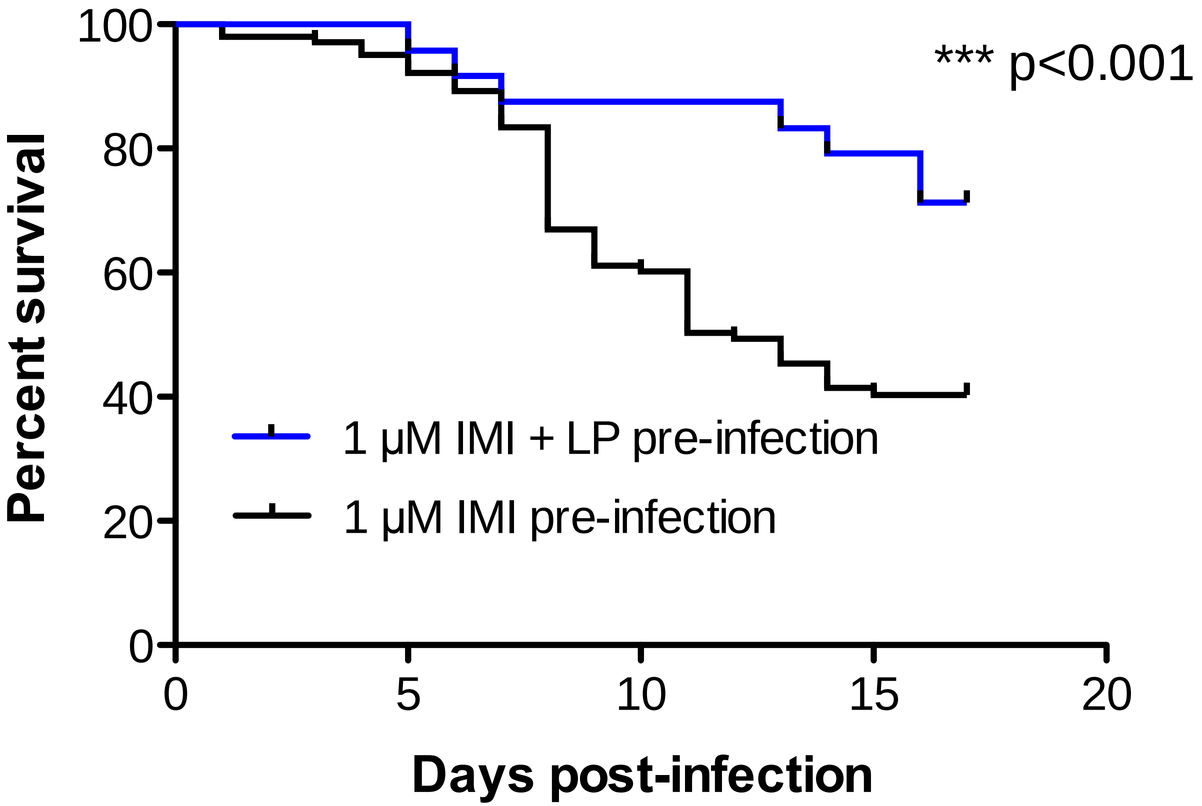


- Lactobacilli can be supplemented onto Honey Bee patties and may offer a simple solution to ameliorate the effects of pesticides

Daisley et al. 2017 Sci. Reports 7: 1-12; Daisley et al. 2018. Appl. Environ. Microbiol. In press.

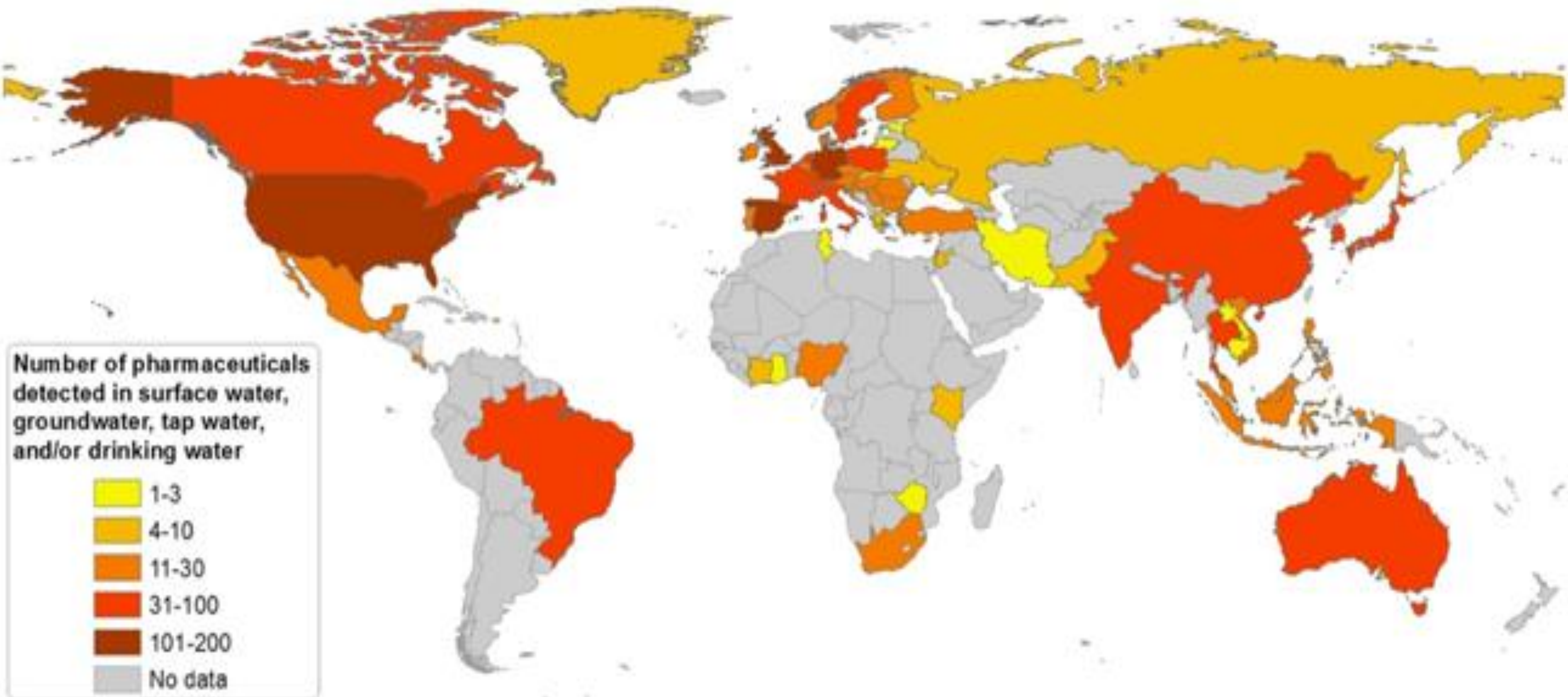
L. plantarum increases survival during imidacloprid/*P. aeruginosa* challenge

- 3 day pretreatment with 1μM imidcloprid



Daisley et al. 2017 Sci. Reports 7: 1-12.

Pharmaceuticals in the environment—Global occurrences and perspectives



You can't make these drugs or the company will sue you, as they OWN them. So, why are they not responsible for these drugs right until they are degraded?

aus der Beek, T et al. 2016

Summary of organic micropollutants studied in Waste Stabilization Ponds

Compound	Average removal efficiency (%)
Stimulants	
Caffeine	70–100
Pharmaceuticals	
Paracetamol	99
Ibuprofen	75–100
Ketoprofen	87–100
Naproxen	75–100
Diclofenac	65–90
Salicylic acid	95–100
Carbamazepine	6–29
Gemfibrozil	15–20
Propranolol	10
Trimethoprim	44–100
Sulfamethoxazole	82–100
Diphenhydramine	85
Found in Personal Care Products	
Methyl dihydrojasmonate	80–99
Galaxolide	75–96
Tonalide	76–96
Cashmeran	82
Furosemide	99
Oxybenzone	99
Methylparaben	81
Triclosan	77–100
Triclocarban	85
Endocrine disrupting compounds	
Octylphenol	71
Bisphenol A (BPA)	24
Androstendione	93–100
Androsterone	100
Eticholanolone	100
17β-Trenbolone	100
Estrone (E1)	31–100
17β-Estradiol (E2)	42–62
Estriol (E3)	26–77
17α-Ethinyl estradiol (EE2)	35–99
4-Nonylphenol (NP)	75
Artificial sweetener	
Sucralose	Not removed
Other	
Benzotriazole	50
5-Methyl benzotriazole	76
Benzothiazole	81
OH-Benzothiazole	86
Tributyl phosphate	59
Tris(2-chloroethyl) phosphate	21
Triphenyl phosphate	76

Y. Gruchlik et al. / Journal of Environmental Management 206 (2018) 202–214

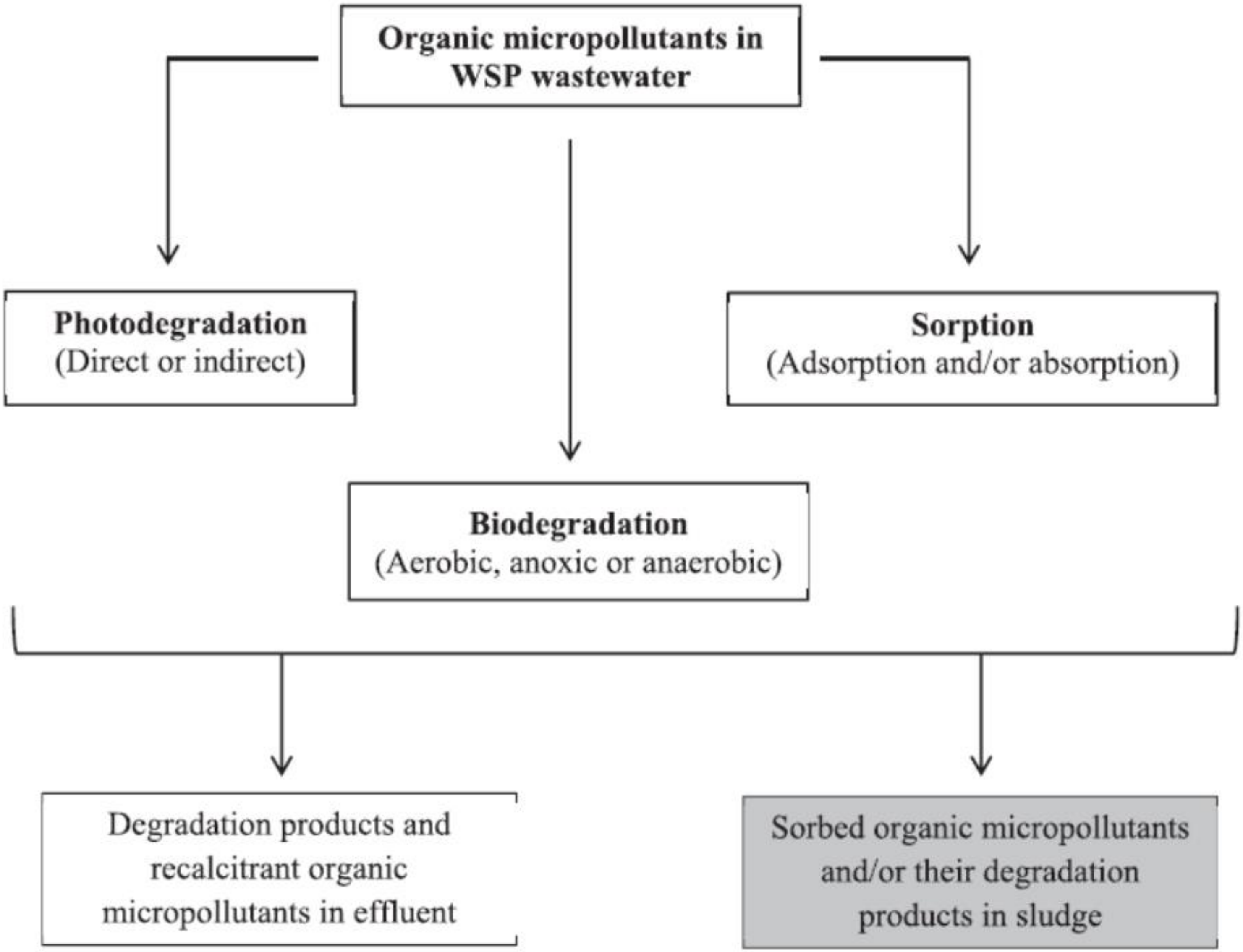


Fig. 1. Removal mechanisms for organic micropollutants in waste stabilisation ponds *Gruchlik Y. et al. J Environ Manage. 2018*

Sci Total Environ. 2010 Mar 1;408(7):1701-6. doi: 10.1016/j.scitotenv.2009.12.012. Epub 2010 Jan 20.

Biodegradation of pharmaceuticals by Rhodococcus rhodochrous and Aspergillus niger by co-metabolism.

Gauthier H[1], Yargeau V, Cooper DG.

Author information

Abstract

This work investigated the possible fate of pharmaceuticals in the environment that are known to be resistant to biodegradation. A co-metabolism approach, adding a readily degradable carbon source, was used to study the biodegradation of some pharmaceuticals. The pharmaceuticals selected were all known to be micro pollutants and frequently used by humans. The microorganisms used primarily were Rhodococcus rhodochrous, known to co-metabolize difficult to degrade hydrocarbons and Aspergillus niger. Because of the long periods of time required for the degradation experiments after growth had reached the stationary phase, it was found to be necessary to correct for water loss from the media. Co-metabolism of carbamazepine, sulfamethizole and sulfamethoxazole was observed and as much as 20% of these compounds could be removed. Small amounts of stable metabolites were observed during the degradation of some of these drugs and these were different from the metabolites obtained from abiotic degradation. A metabolite arising from the biodegradation of sulfamethoxazole by R.rhodochrous was identified.

The bottom line

- We have created a huge chemical imprint on a planet that is, at its core, microbial. We need to better use microbes to offset the negative effects of these chemicals.
- This includes crop and livestock production, waste water treatment, air pollution, fish farming, mining and food preservation.
- The use of probiotics, or beneficial microbes if they do not meet the probiotic definition, has enormous potential across our ecosystem.

We can’t consider health in isolation