The MERCURY-CLEARING EFFECT of *Sargassum sp.* SEAWEED in MERCURY-CONTAMINATED WATER as MEASURED by COLD VAPOR ATOMIC ABSORPTION SPECTROPHOTOMETRY

A 2x3 Prospective Randomized Controlled Lab Trial

By

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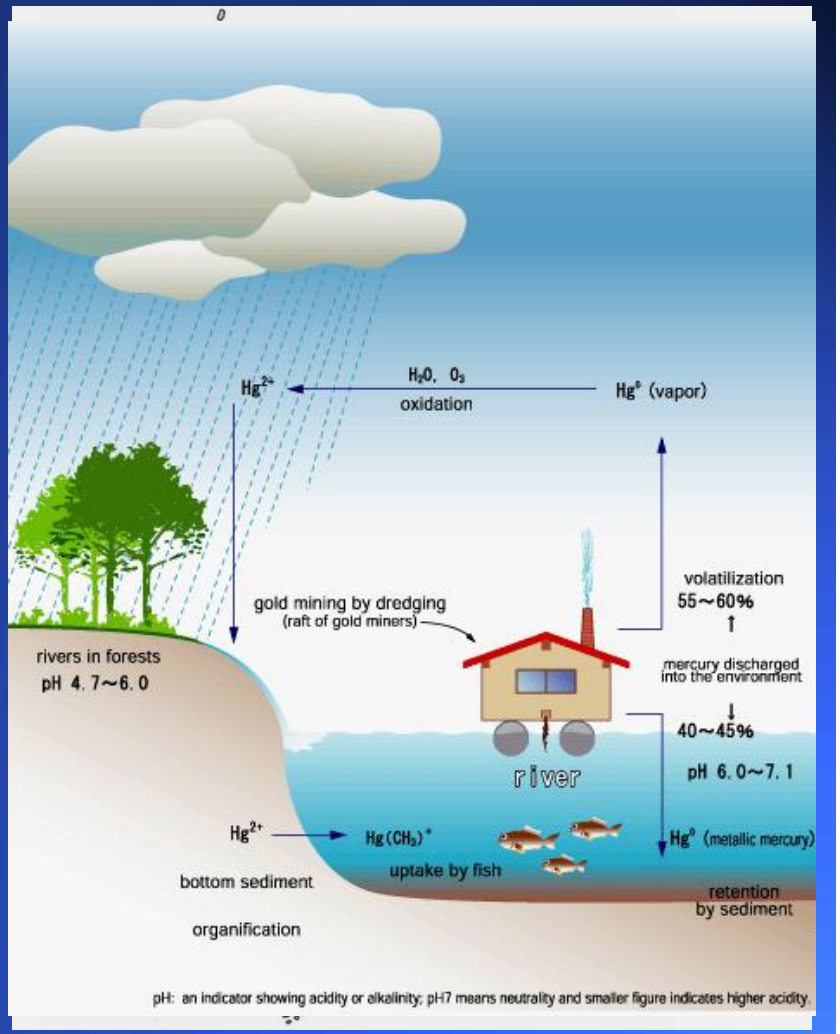
Cebu Doctors' University, College of Medicine, 2009

Background on Mercury Pollution

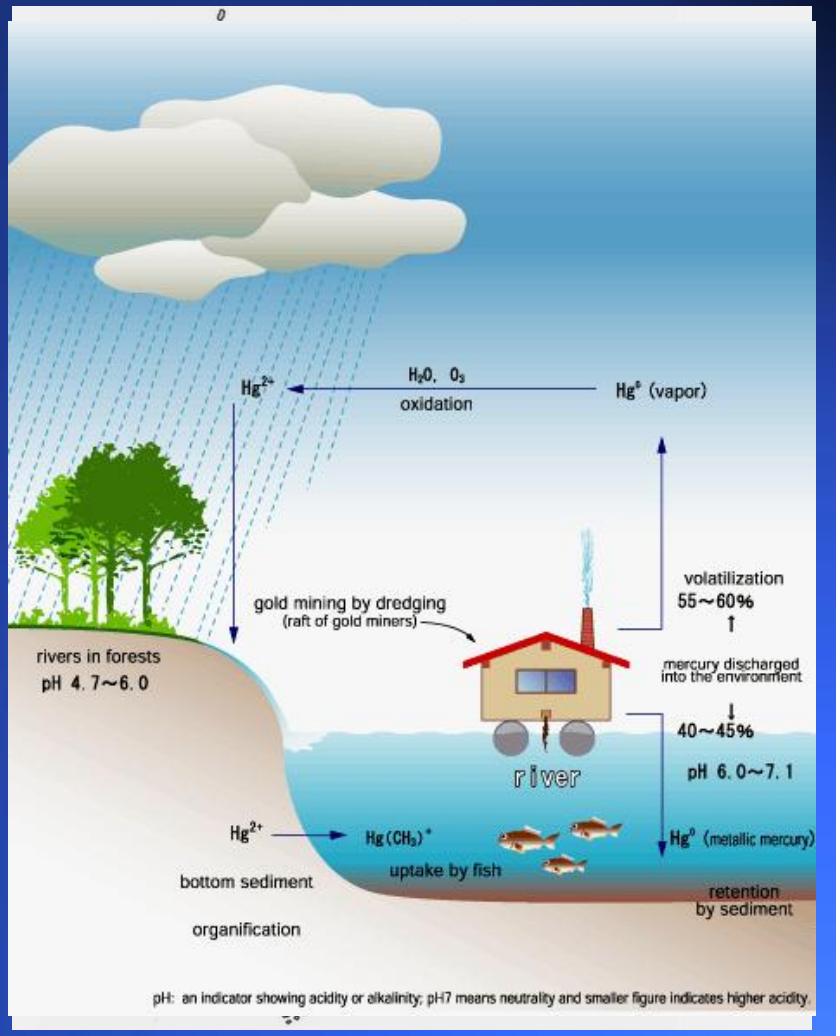
- ✓ In the Philippine setting, Hg pollution is largely ass ociated with Gold mining.
- ✓ Hg leaks into the envir onment due to faulty handling or intentional disposal into waterways.
- ✓ Hg discharged into waterwa ys is transformed into methyl-Hg, which is eaten by edible aquatic species, or is absorbed by humans directly f rom contaminated drinking water.



Danilo C. Israel and Jasminda P. Asirot. "Mercury Pollution Due to Small-Scale Gold Mining in the Philippines." Philippine Institute For Development Studies, 2002.

Background on Mercury Pollution

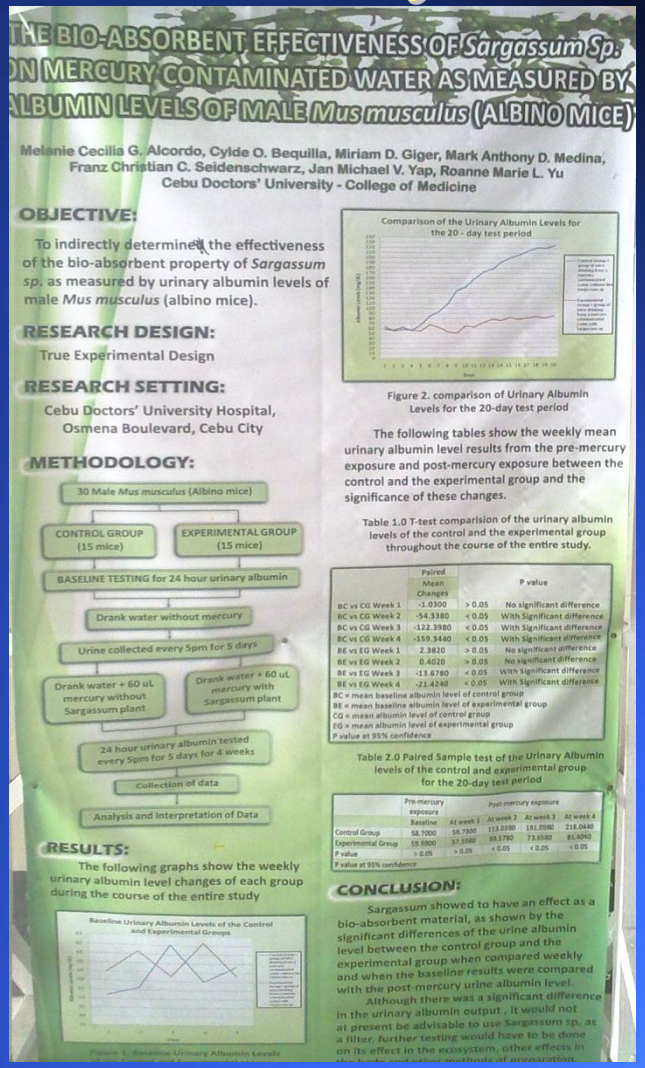
- ✓ Hg water pollution levels in the Philippines **were found to be beyond the 2 εg / Liter limit** in filtered water samples in some monitoring locations.
- ✓ **~ 13.5 metric tons of toxic Hg is washed yearly into major rivers**
  - é from Naboc to Kinking,.. into the Davao Gulf.
  - é Hg from Compostela Valley drain into Butuan Bay.
  - é Hg-stained stream sediments also threaten the Agusan River.



***GreenPeace. "The State of Water Resources* in the Philippines." October 2007.**

Background on Sargassum Study

- ✓ Alcordo *et al* (CDU 2008-09) found experimental & field basis for using Sargassum seaweed in clearing heavy metals from water.
- ✓ A promising animal lab trial was performed, showing potential effects of Sargassum treament of Hg - contaminated water.
- ✓ Methodological setbacks & pitfalls detracted from the applicability of this study ï hence the decision to upgrade the entire study the following year.



THE BIO-ABSORBENT EFFECTIVENESS OF *Sargassum Sp.* ON MERCURY CONTAMINATED WATER AS MEASURED BY ALBUMIN LEVELS OF MALE *Mus musculus* (ALBINO MICE)

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OBJECTIVE:
To indirectly determine the effectiveness of the bio-absorbent property of *Sargassum sp.* as measured by urinary albumin levels of male *Mus musculus* (albino mice).

RESEARCH DESIGN:
True Experimental Design

RESEARCH SETTING:
Cebu Doctors' University Hospital, Osmena Boulevard, Cebu City

METHODOLOGY:
30 Male *Mus musculus* (Albino mice)
CONTROL GROUP (15 mice) | EXPERIMENTAL GROUP (15 mice)
BASELINE TESTING for 24 hour urinary albumin
Drank water without mercury
Urine collected every 5pm for 5 days
Drank water + 60 uL mercury without Sargassum plant | Drank water + 60 uL mercury with Sargassum plant
24 hour urinary albumin tested every 5pm for 5 days for 4 weeks
Collection of data
Analysis and Interpretation of Data

RESULTS:
The following graphs show the weekly urinary albumin level changes of each group during the course of the entire study

Baseline Urinary Albumin Levels of the Control and Experimental Groups

Comparison of the Urinary Albumin Levels for the 20 - day test period

Figure 2. comparison of Urinary Albumin Levels for the 20-day test period

The following tables show the weekly mean urinary albumin level results from the pre-mercury exposure and post-mercury exposure between the control and the experimental group and the significance of these changes.

Table 1.0 T-test comparision of the urinary albumin levels of the control and the experimental group throughout the course of the entire study.

	Paired Mean Changes	P value	
BC vs CG Week 1	-1.0300	> 0.05	No significant difference
BC vs CG Week 2	-54.3380	< 0.05	With Significant difference
BC vs CG Week 3	-122.3980	< 0.05	With Significant difference
BC vs CG Week 4	-159.3440	< 0.05	With Significant difference
BE vs EG Week 1	2.3820	> 0.05	No significant difference
BE vs EG Week 2	0.4020	> 0.05	No significant difference
BE vs EG Week 3	-13.6780	< 0.05	With Significant difference
BE vs EG Week 4	-21.4240	< 0.05	With Significant difference

BC = mean baseline albumin level of control group
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P value at 95% confidence

Table 2.0 Paired Sample test of the Urinary Albumin levels of the control and experimental group for the 20-day test period

	Pre-mercury exposure	Post-mercury exposure			
	Baseline	At week 1	At week 2	At week 3	At week 4
Control Group	58.7000	58.7300	113.0380	181.0980	218.0440
Experimental Group	59.9800	57.5980	59.1780	73.6580	81.4040
P value	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05

P value at 95% confidence

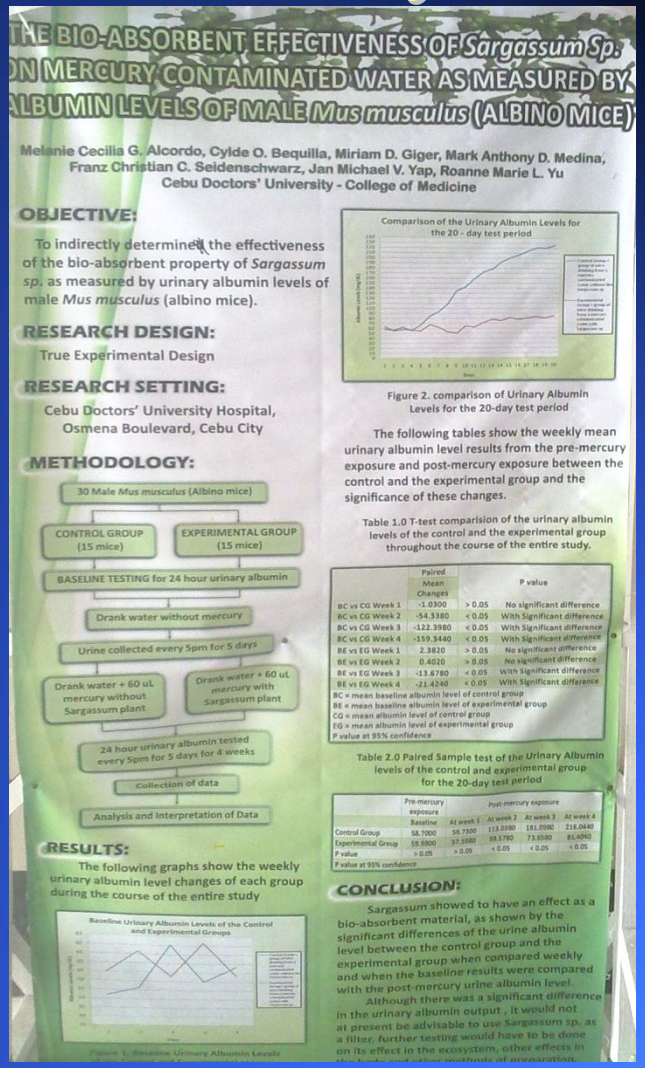
CONCLUSION:
Sargassum showed to have an effect as a bio-absorbent material, as shown by the significant differences of the urine albumin level between the control group and the experimental group when compared weekly and when the baseline results were compared with the post-mercury urine albumin level. Although there was a significant difference in the urinary albumin output , it would not at present be advisable to use Sargassum sp. as a filter, further testing would have to be done on its effect in the ecosystem, other effects in

Alcordo MC et al. "The Bio-absorbent Effectiveness of Sargassum sp. On Mercury-contaminated Water, as measured by Albumin Levels of Male Mus musculus (Albino Mice). CDU College of Medicine, Cebu Doctors' Uiversity, May 2009.

Background on Sargassum Study

OUR MAIN GOALS? é

- ✓ é Re-visit & ***adequately answer*** the original research question;
- ✓ é Use ***valid outcome measures*** ;
- ✓ é Use an ***adequately powered*** study design;
- ✓ é Eliminate / control ***confounders***;
- ✓ é Teach & apply *CORRECT* ***statistical research principles*** ;
- ✓ ***…Do clinically relevant, student-level, consultant-driven research work !***



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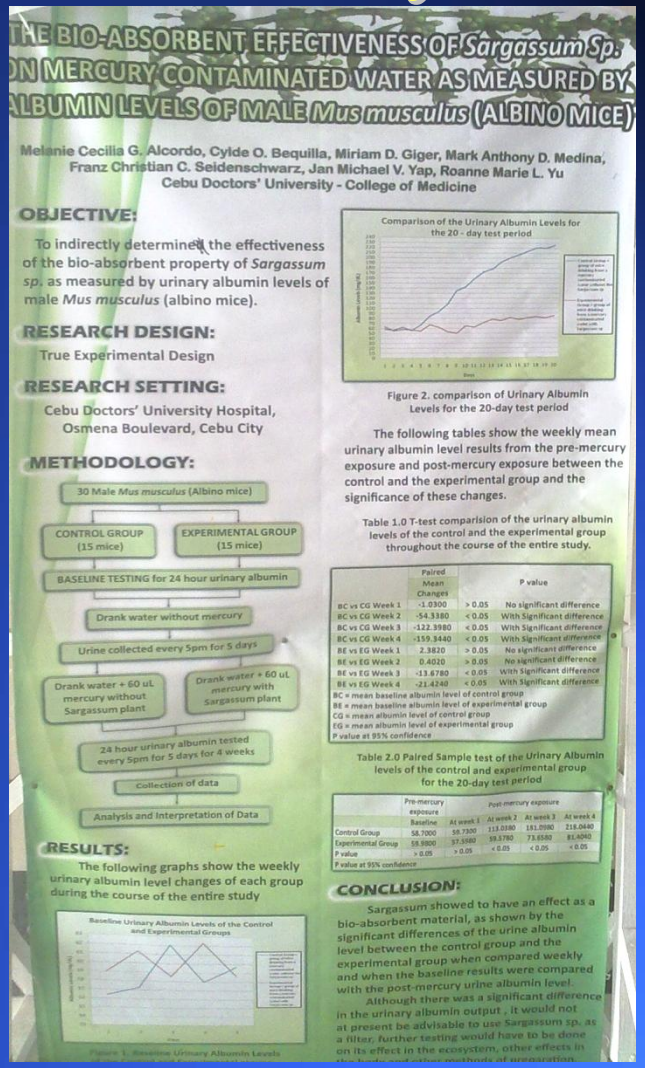
Background on Sargassum Study

- ✓ **Can Sargassum seaweed lower Hg content of contaminated water?**
- ✓ Instead of an *INDIRECT APPROACH* at measurement (e.g., an animal study), why not measure contaminated water itself?.. And DIRECTLY COMPARE post-treatment Hg levels of water samples!
- ✓ Processed Sargassum sp. showed BIO-ABSORBENCY properties on other heavy metals like Cd, Co, Cr & Al (*Voelkel, 2001; Davis, 2003 & 2004*).



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Sargassum Bio-absorbency Study

- ✓ Distilled water ñstockò = 4 Liters.
- ✓ Original 4-liter stock was divided into TWO 2-liter treatment stocks.
- ✓ Contamination was achieved using elemental Hg → [> 2 εg / Liter].
- ✓ The treatment stock assigned for experimental exposure was then given 6 grams of dried Sargassum weed after an hour of Hg contamination.
- ✓ Fifteen 100 mL *random-sequence* samples were taken from each of the 2-liter stocks (N=30, 15 per group).

Sargassum Bio-absorbency Study

4 Liters H2O Stock

2 Liters H_2O Stock

2 Liters H_2O Stock

2L H_2O + Hg

2L H_2O + Hg + Sarg

5 samples on DAY 1

5 samples on DAY 2

5 samples on DAY 3

5 samples on DAY 1

5 samples on DAY 2

5 samples on DAY 3

CV AAS on DAY 1

CV AAS on DAY 2

CV AAS on DAY 3

CV AAS on DAY 1

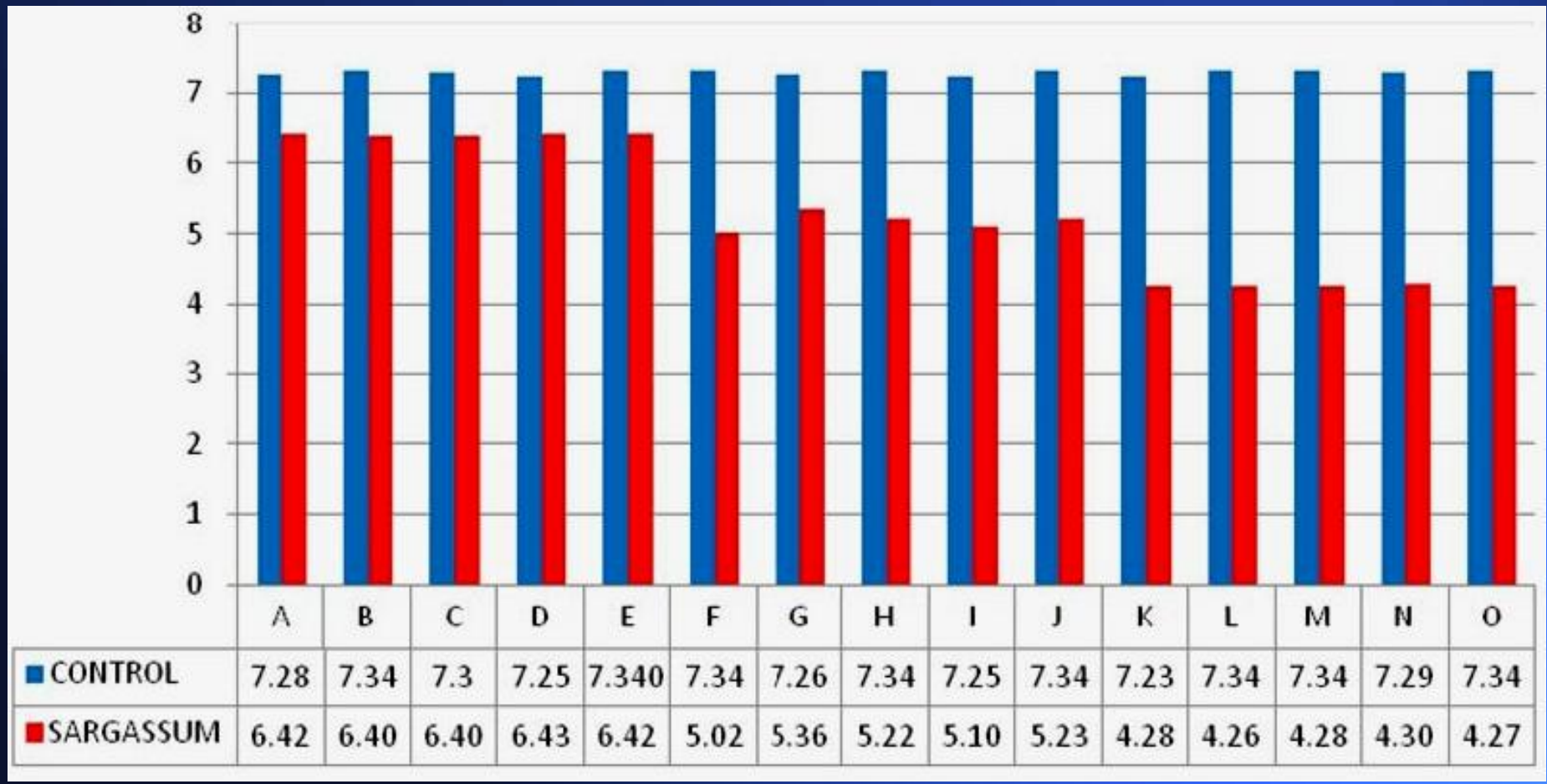
CV AAS on DAY 2

CV AAS on DAY 3

STATISTICAL METHODS: 2x3 ANOVA w/ all pair-wise adjusted t-Tests & *post-run* power analyses

Sargassum Bio-absorbency Study

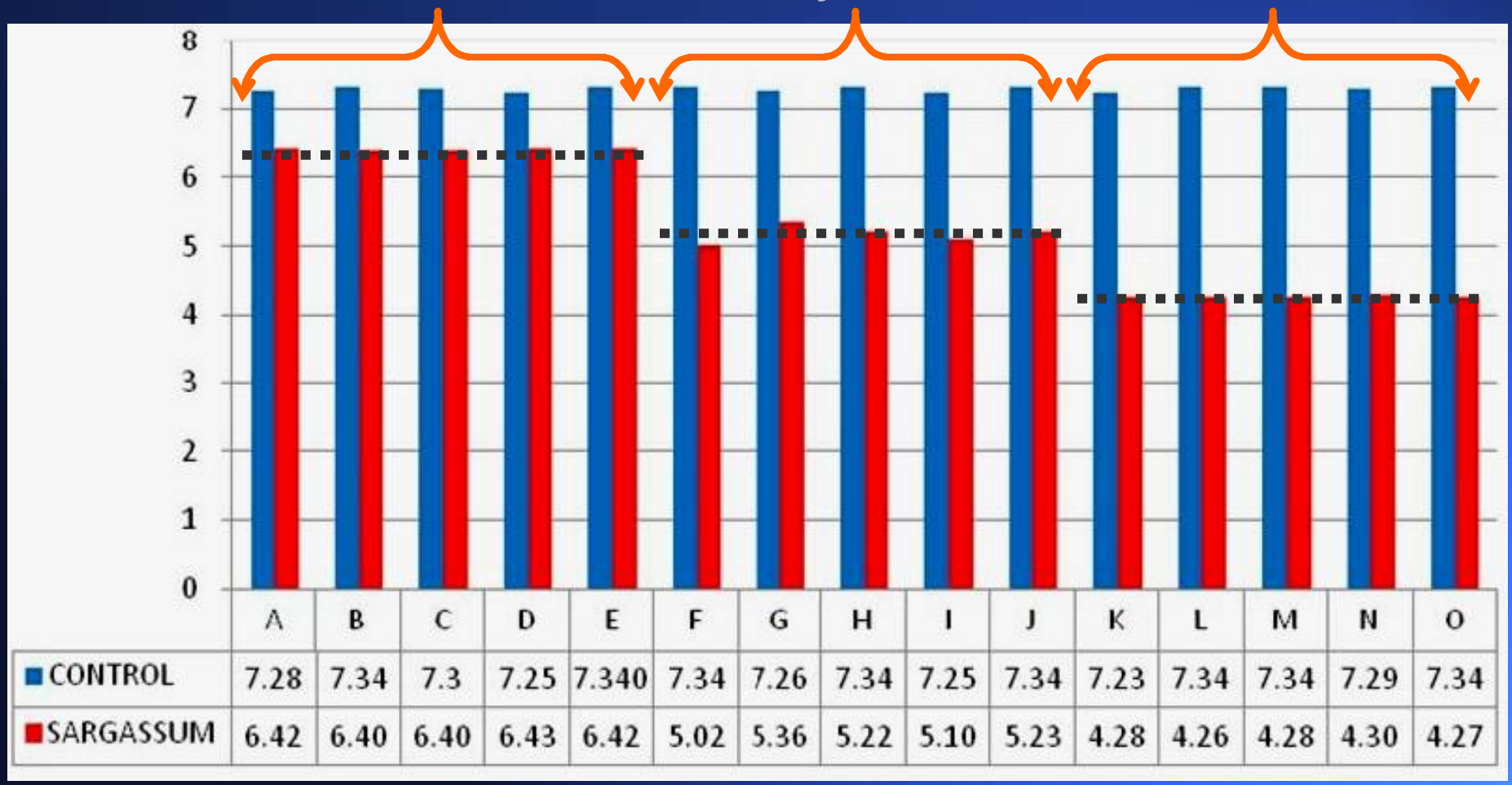
- ✓ Testing of each 100 mL sample (N=30)
- ✓ Original plan was a one-day run.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
CONTROL	7.28	7.34	7.3	7.25	7.340	7.34	7.26	7.34	7.25	7.34	7.23	7.34	7.34	7.29	7.34
SARGASSUM	6.42	6.40	6.40	6.43	6.42	5.02	5.36	5.22	5.10	5.23	4.28	4.26	4.28	4.30	4.27

Sargassum Bio-absorbency Study

- ✓ Testing of each 100 mL sample (N=30)
- ✓ Lab's schedule conflicts → 3-day run!



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Sargassum Bio-absorbency Study

- ✓ In order to remove the confounding effect of TIME OF RUN ...
- ✓ Convert ñTIMEòinto a second independent variable!

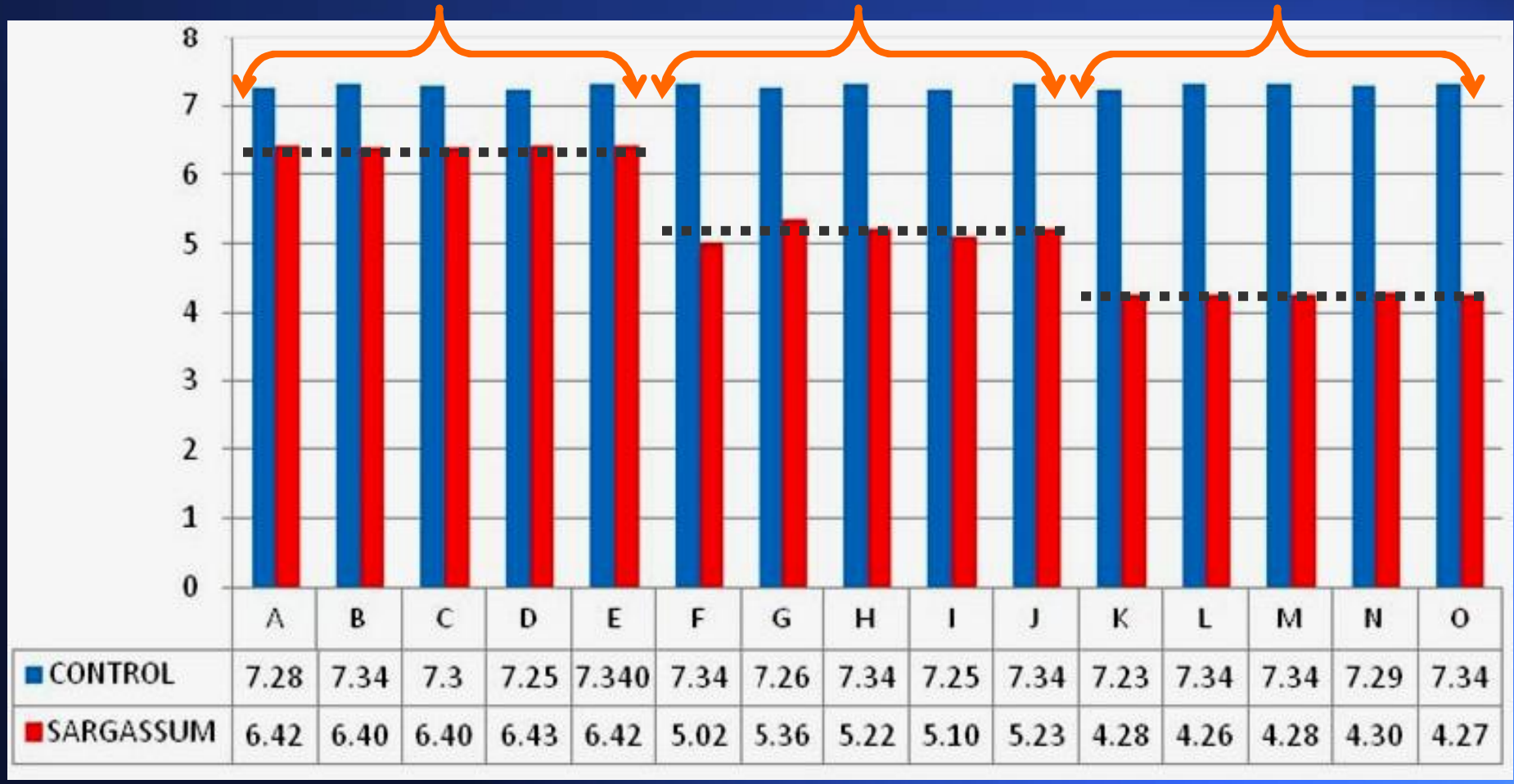

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Table 2B. Pair-wise Comparison for Day of Testing*

Group	# of samples	Mean Hg (µg/L)	this group is significantly different from …
Batch Day #1	10	6.877	*Batch Day #2, Batch Day #3*
Batch Day #2	10	6.308	*Batch Day #1, Batch Day #3*
Batch Day #3	10	5.809	*Batch Day #1, Batch Day #2*

*significant at α=0.05

Sargassum Bio-absorbency Study

- ✓ Dried Sargassum seaweed is a viable means of clearing Hg-contaminated water (via BIO-ABSORBENCY).

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*significant at α=0.05

Sargassum Bio-absorbency Study

- ✓ Hg-clearing effect of Sargassum is TIME-dependent ï i.e., the longer the exposure, the greater the Hg clearance.

For comments, correspondence & requests for Research Methodology-related Literature, please write or visit us at…

Cebu Medical Research Online

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