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Effect of Flood in Chlorophyll *"a"* Concentration in the River

Introduction:

Chlorophyll "a" is a light-absorbing pigment used by plants for photosynthesis. The algal community is the primary source of this parameter in the water, some algae are suspended in the water and some are attached in a substrate. This parameter is an estimate on the standing algal biomass.

This short-term research aims to measure the effect of flood on Chlorophyll "a" concentration and algal biomass in the river.

Kuro-Kawa in Kiso-Fukushima, was selected as the study site before and after the flood. First collection of samples was done on August 10, 2014 and second collection was on August 13, 2014.

Sampling Procedure:

- Identification of the sampling point based on the samples needed for your research.
- After identifying the sampling site, record the physic-chemical data using in-situ equipments and other physical observations.
- Collect five cobbles from the river bed, use the top portion of the rock and place 6cm x 6cm rubber frame to make a guide and by using a toothbrush slowly removes the algae attach to the rock then wash well using a collecting tray.
- Samples were then placed in a container with ice before being brought to laboratory.

Laboratory Analysis Procedure:

- Prepare vacuum filtration system (suction bottles, filtering apparatus, hand-pump and 47mm glass fiber filter).
- Record the total volume of samples before filtration. Adjust the volume of samples to be filtered depending on the concentration (stop before the filter clogs), record the volume of the filtered samples.

- After filtration, place the glass fiber filter in paper towel to dry. Cut the filter into small pieces (3-4mm width) and place in a flask. Add 15ml 90% acetone. Cover the flask with parafilm and placed it in cool and dark place (coolers) overnight.
- Put filter paper (no. 5, 90mm) on a funnel and filter the samples to remove the cut glass fiber and put into a test tube.
- Measure the absorbance of the samples at 750, 663, 645 and 630nm using a spectrophotometer. Used 90% acetone as a control. Since absorbance at 750nm is the turbidity measurement, subtract this value from all other measurements. Once measured, put the solution back into the test tube. Calculate total Chlorophyll "a" concentration using the formula one. (Unesco Method)
- Measure absorbance at 750 and 665nm then add 2 drops of IN HCl to the solution and leave for a few minutes before re-measuring. Calculate active Chlorophyll "a" concentration and Pheophythin "a" using formula 2 and 3 respectively.
- Formula 1 – 3 show the amounts of Chlorophyll "a" and Pheophythin "a" in 1ml of acetone extract.

    - Formula 1^{A} : Total (Chl "a") (µg/ml) = $11.64*E_{663} - 2.16*E_{645} + 0.10*E_{630}$.
      [A] The values E_{663}, E_{645} and $*E_{630}$ are the absorbance measured at 663, 645 and 630 after subtraction by that at 750nm. i.e. absorbance measurement without turbidity measurement.
    - Formula 2^{B} : Active (Chl "a") (µg/ml) = $26.7*(E_{665} - E_{665}a)$
      [B] E_{665} is the absorbance measured at 665 nm minus that at 750nm. $E_{665}a$ is the absorbance measured at 665nm after HCl addition minus that at 750nm.
    - Formula 3^{C} : Pheophythin "a") (µg/ml) = $26.7*(1.7*E_{665}a - E_{665})$
      [C] Pheophythin "a" is a chlorophyll molecule broken down to lose 2 Mg^{2+}. High Pheophythin "a" indicates low algal activity. i.e. many dead/weak cells.

- From the area scraped for algae and amount of water filtered, convert the Chlorophyll "a" into mg/m^2.

    - Algal Biomass (mg Chl "a"/m2) = 1/1000*Chlorophyll "a" * amount of extract*(re-suspension/filtered water)*1/scraped area (5 stones with area of 6cm x 6cm.

Results:

1. Environmental Assessment of Sampling Point

Date	Time	Site	Habitat	pH	Conductivity (mS/m)	D.O. mg/L	Temp.	Light Intensity (micro mol/sec/m2)	Notes/ Remarks
Aug. 10, 2014	10:30 AM	Kuro-Kawa	Pool	7.85	8	8.54	16.53	267.9	Low precipitation and cloudy
	9:26 AM	Kuro-Kawa	Riffle	7.4	7	8.51	16.46	94.37	High precipitation and very cloudy
Aug. 13, 2014	9:10 AM	Kuro-Kawa	Pool	7.59	4	9.23	14.8	1540.3	Sunny, water level is high
	10:30 AM	Kuro-Kawa	Riffle	7.72	3	9.27	14.6	58.50	Water current is very strong

2. Photos of Sampling Points

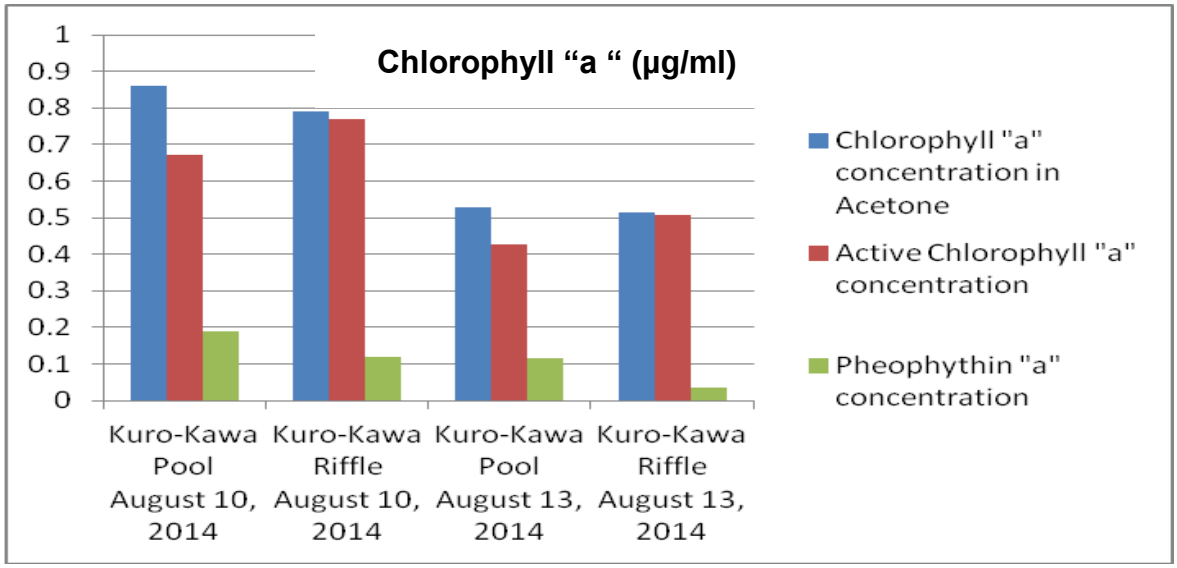
Kuro-Kawa Pool, August 10, 2014

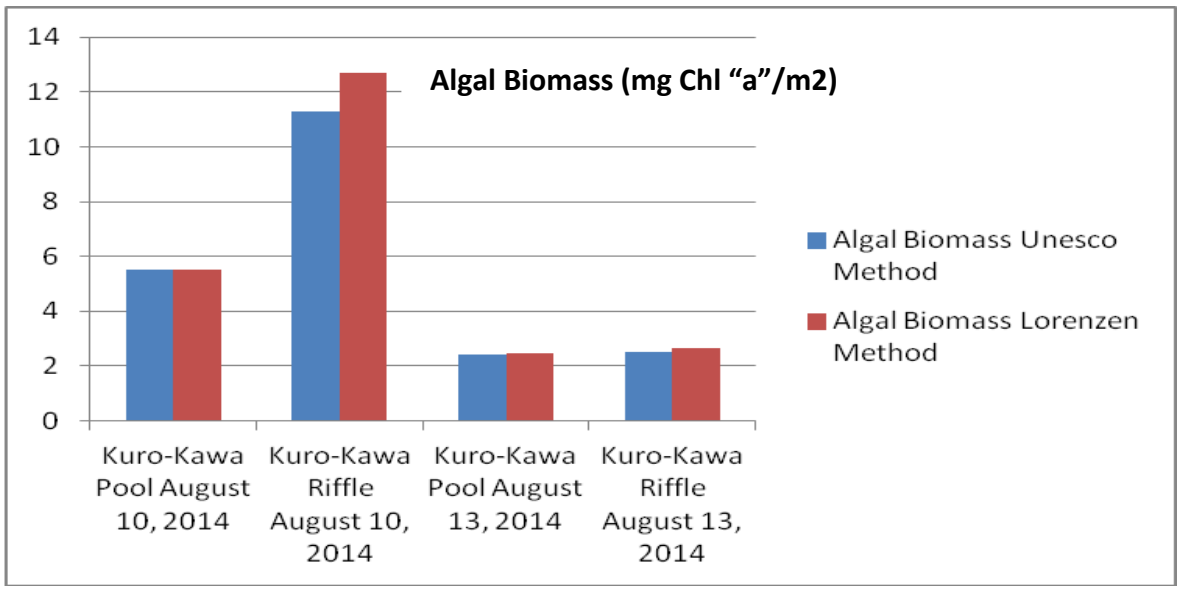
Kuro-Kawa Pool, August 13, 2014

Kuro-Kawa Riffle, August 10, 2014

Kuro-Kawa Riffle, August 13, 2014

3. Comparison of Laboratory Results Using Unesco Method and Lorenzen Method (Before and After the Flood)





	Unesco Method			Lorenzen Method			
	Chlorophyll "a" concentration (µg/ml)	Algal Biomass	Remarks	Active Chlorophyll "a" concentration (µg/ml)	Pheophythin "a" concentration (µg/ml)	Algal Biomass	Remarks
August 10, 2014				**August 10, 2014**			
Kuro-Kawa Pool	0.86	5.5		0.67	0.19	5.5	
Kuro-Kawa Riffle	0.79	11.3		0.77	0.12	12.7	
August 13, 2014				**August 13, 2014**			
Kuro-Kawa Pool	0.53	2.41	38.37% decreased in Chl "a"	0.43	0.12	2.47	35.82% decreased in Active

			and 56.18% decreased in Algal Biomass				Chl “a” and 55.09% decreased in Algal Biomass
Kuro-Kawa Riffle	0.52	2.51	34.18% decreased in Chl “a” and 77.79% decreased in Algal Biomass	0.51	0.04	2.64	33.77% decreased in Active Chl “a” and 79.21 % decreased in Algal Biomass

Results/Conclusion:

Comparing the data obtained between the result of analysis on August 10 and August 13, 2014, it was revealed that both Chlorophyll “a” concentration and Algal Biomass decreased after the flood. This in effect shows that the algae attached or present in the substrate could have been wiped out due to flooding.

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