

## **SHORT – TERM RESEARCH**

**DIWPA International Field Biology Course – Kiso River, August 9 to 16, 2014**

**Center for Ecological Research – Kyoto University**

# **Effect of Flood in Chlorophyll “a” Concentration in the River**

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## Introduction

Chlorophyll “a” is a light-absorbing pigment used by plants for photosynthesis. The algal community is the primary source of these parameters, some algae are suspended in the water and some are attached in a substrate. This parameter is used to estimate the algal biomass and also to predict the algal Bloom.

This short-term research will try to measure the effect of flood in Chlorophyll “a” concentration and algal biomass in the river.

Kuro-Kawa in Kiso-Fukushima, was selected to conduct monitoring and sampling before and after the flood. First collections of samples were done on August 10, 2014 and second collection was on August 13, 2014.

## Sampling Procedure:

Please identify first the sampling point based on the samples you need to collect for your research.

After identifying the sampling site, record the physico-chemical data using in-situ equipments and other physical observations.

Collect five pieces of cobbles from the river bed and bring it to the side of the river to collect the sample properly.

Use the top portion of the rock and place the 6cm x 6cm rubber frame to make a guide, then remove algae from the rocks using toothbrush, wash well in a collecting tray.

Put the composite samples in a container, then placed it in a cooler and bring it back to the laboratory.

Note: Before going the site check all the equipment and materials essential to sampling and always bring a sampling guide to avoid troubles.

## Procedure on Laboratory Analysis:

In the laboratory, prepare vacuum filtration system (suction bottles, filtering apparatus, hand-pump and 47mm glass fiber filter).

Record the total volume 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.

Place filter paper (no. 5, 90mm) on a funnel and filter the samples to remove the cut glass fiber and put into a test tube.

Use Unesco method and Lorenzen Method to measure the Chlorophyll "a" concentration and the total Algal Biomass

Formula 1<sup>A</sup>: Total (Chl "a") ( $\mu\text{g}/\text{ml}$ ) =  $11.64 * E_{663} - 2.16 * E_{645} + 0.10 * E_{630}$ .

Formula 2<sup>B</sup>: Active (Chl "a") ( $\mu\text{g}/\text{ml}$ ) =  $26.7 * (E_{665} - E_{665a})$

Formula 3<sup>C</sup>: (Pheophythin "a") ( $\mu\text{g}/\text{ml}$ ) =  $26.7 * (1.7 * E_{665a} - E_{665})$

<sup>A</sup> The values  $E_{663}$ ,  $E_{645}$  and  $E_{630}$  are the absorbance measured at 663, 645 and 630nm after subtraction by that at 750nm. i.e. absorbance measurement without turbidity measurement.

<sup>B</sup>  $E_{665}$  is the absorbance measured at 665nm minus that at 750nm.  $E_{665a}$  is the absorbance measured at 665nm after HCl addition minus that at 750nm.

<sup>C</sup> Pheophythin "a" is a chlorophyll molecule broken down to lose 2  $\text{Mg}^{2+}$ . 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  $\text{mg}/\text{m}^2$ .

Algal biomass ( $\text{mg Chl "a"}/\text{m}^2$ ) =  $1/1000 * \text{Chlorophyll "a"} * \text{amount of extract} * (\text{re-suspension/filtered water}) * 1/\text{scraped area (5 stones with area of } 6\text{cm} \times 6\text{cm)}$ .

# Results

## 1. Environmental Assessment of Sampling Point

Date of collection	Time	Site	Habitat	pH	Conductivity (mS/m)	D.O. mg/l	Temperature	Light intensity (micro mol/sec/ m <sup>2</sup> )	Notes/Remarks
<b>August 10, 2014</b>	10:30	Kuro-Kawa	Pool	7.85	8	8.54	16.53	267.9	Low precipitation and cloudy
	9:26	Kuro-Kawa	Riffle	7.4	7	8.51	16.46	94.37	High precipitation and cloudy
<b>August 13, 2014</b>	9:10	Kuro-Kawa	Pool	7.59	4	9.23	14.8	1540.3	Sunny, water level is high
	10:30	Kuro-Kawa	Riffle	7.72	3	9.27	14.6	58.50	Water current is very strong

## 2. Photos of Sampling Points



Pool



August 13, 2014

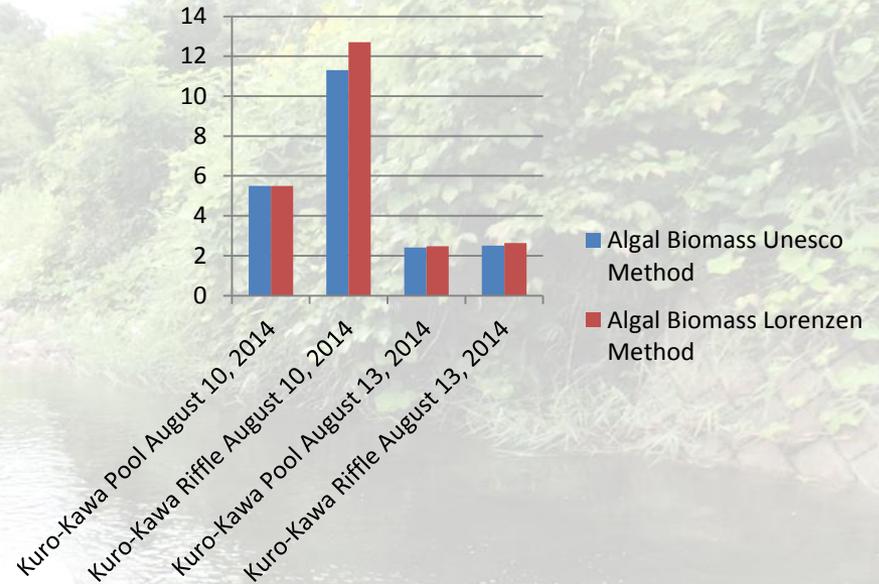
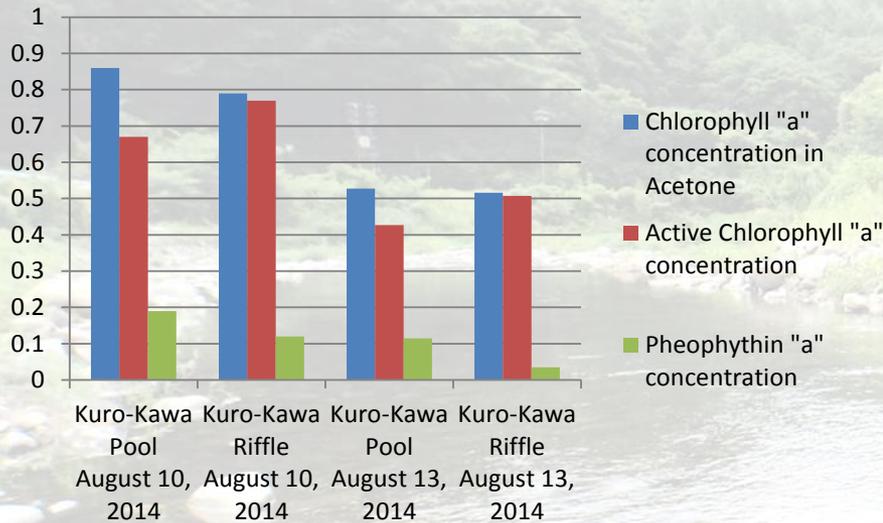
August 10, 2014



Riffle



### 3. Comparison of laboratory results using Unesco Method and Lorenzen Method (Before and After the Flood)



	Using Unesco Method		Using Lorenzen Method		
	Chlorophyll "a" concentration	Algal Biomass	Active Chlorophyll "a" concentration	Pheophytin "a" concentration	Algal Biomass
<b>August 10, 2014</b>					
<b>Kuro-Kawa Pool</b>	<b>0.86</b>	<b>5.5</b>	<b>0.67</b>	<b>0.19</b>	<b>5.5</b>
<b>Kuro-Kawa Riffle</b>	<b>0.79</b>	<b>11.3</b>	<b>0.77</b>	<b>0.12</b>	<b>12.7</b>
<b>August 13, 2014</b>					
<b>Kuro-Kawa Pool</b>	<b>0.527</b>	<b>2.406</b>	<b>0.427</b>	<b>0.115</b>	<b>2.473</b>
<b>Kuro-Kawa Riffle</b>	<b>0.516</b>	<b>2.512</b>	<b>0.507</b>	<b>0.035</b>	<b>2.641</b>

## Discussion

1. There are limitations in this study which may contribute to the research error:

Bad weather condition can cause problem in collecting the samples that may affect the result of analysis.

Time (short/limited)

2. The result shows that Chlorophyll “a” concentration and Algal Biomass were decrease with an average of 55.65% in Kuro pool and 78.49% in Kuro Riffle after the flood. There are reasons to consider, why the algal biomass decreases after the flood.

Algae that attached in a substrate wiped out by the flood as well as the algae that are suspended in the water.

Maybe the stones that were collected was only drift by flood came from other place, and the top side of the rock might be the bottom side from its original location.

## Findings

In this research it is clear that the concentration of Chlorophyll “a” decreases after the flood. It is possible that Chlorophyll “a” concentration can directly affected by the flood, but now it is just an assumption

To get a concrete data about this research it should be repeated several times to compare the result.



**Thank you very much!**

