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INFLUENCE OF CHROMIUM METAL ON CHLOROPHYLL CONTENT IN LEAVES OF PADDY *Oryza sativa* L

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ABSTRACT

The aim of the procedure undertaken in 2014 was to calculate the concentration of Chlorophyll a, Chlorophyll b, total chlorophyll (a and b) and Chlorophyll (a/b) ratio in five ages (17, 47, 77, 107, 145) days from planting date, in leaf of Paddy that's was grown in three sites has different concentration of Chromium., two fields (field 1 and 2) near the ultrabasic soil are located in Ranau, Sabah, and the other field (Field 3) taken as control is at the UKM experimental plot Paddy field in peninsular of Malaysia for the year 2014. The plant species used in the present investigation is Sarwak merah. The intention was to evaluate the effects of Chromium in conjunction with the relative age of the plant. The output parameter were sensitive to increase the Chromium concentration and age of paddy plant, the result showed significant differences in sites and age of Paddy plant, The most dramatic effect on both types of Chlorophyll, a and b and by extension therefore, total Chlorophyll was at the Ranau sites, which has high levels of Chromium specially Ranau field 1. While the lowest effects were recorded at the UKM experimental plot Paddy field in peninsular of Malaysia. Chlorophyll a, Chlorophyll b and total chlorophyll (a and b) declined progressively with increasing concentrations of chromium and age of Paddy plant, while the Chlorophyll (a/b) ratio was increased slightly with increasing chromium concentration and age of plant also.

Key words: Chlorophyll content, Chromium, Paddy.

INTRODUCTION

The largest contaminant of soils by heavy metal deposits is sewage sludge closely followed by the detritus left as a result of mining for metal ores[1]. The disastrous impact on the environment as a consequence of mining and its dumping of waste products on the

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surrounding landscape is well documented and clearly observable[2,3]. The negative consequences of mining are mainly due to the deposits or 'tailings' that are left behind them[4]. These 'tailings' often have characteristics that make them unsuitable, indeed hostile, to normal vegetative growth. These characteristics include low pH[5], high concentrations of toxic metals[5-7], little capacity to retain water and little in the way of essential plant nutrients[8]. In addition, tailings are often steeply graded, resulting in instability and consequent erosion. These are all causal factors leading to degradation, not only in the soil, but also in the water supply both at ground level and subterranean. Heavy metals in particular do not degrade rapidly and will continue to contaminate both soil and water for a very considerable time span[9]. Some of these heavy metals, such as chromium, constitute a health hazard to human life. An array of human activities, including the manufacture and use of metal-containing pesticides, the day-to-day running of power plants, the production of metals such as iron and steel, the mining of metals in particular, the electroplating of metals, the manufacture of pigments and the preserving of timber among many others can cause the ground to become contaminated with heavy metal residues. Increased chromium levels in plants impacts on biological factors, finally resulting in a complete loss of growth and the land becoming infertile[10]. Chromium contamination has therefore become a source of increasing concern in recent times. Biological symptoms of Chromium contamination include failure to germinate, poor growth, pale/yellow vegetation (chlorosis) and overall marked decrease in plant size[11]. Chromium is not essential for plant growth and is highly toxic[12]. The impacts of chromium on photosynthesis, pigmentation, nitrate activity and protein content has shown toxicity in some algae[13]. Chromium has not only been found to be harmful to vegetation but when present in food crops it readily gains access to the food chain and thence to humans. Whilst, it has been shown that differing plant species have different reactions to the toxicity caused by heavy metals, some plants managing to survive on contaminated soils and not accumulate metals, there are others that take up the metals, tolerate them and accumulate them at a higher rate. These are known as hyper-accumulators. The way that these metals interact with the plant's cells can cause a variety of responses inevitably leading to a change in the developmental growth of the organism[14].

It is proven for example that chromium is a causal factor in both pale/yellow leaves (chlorosis) and cellular death (necrosis) in plants, and increased levels in the overall environment can have a detrimental effect on various organisms[15]. However, other heavy metals, at relatively low levels can actually increase the level of biological activity[16]. It has also been shown[17] that the degree of contamination by heavy metals depends on a variety of other environmental factors such as the composition of the soil in terms of organic material, ferrous and manganese oxides, clay and other minerals, all of which affect the mobility of the heavy metals, their consequent uptake, release and relationship with other components of the environment such as vegetative growth[17]. Chromium has proved to be an important component in our environment, usually being found at oxidation rates of (+ III and + VI)[18] and this study was conducted in order to observe and note the effects of sites with differing levels of chromium toxicity and its subsequent effects on the levels of chlorophyll found in the leaves of a particular plant at various stages in its development.

EXPERIMENTAL

Materials and methods

Soil analysis

The Paddy plants and soil were obtained from three areas, two fields in Ranau, Sabah (Field 1 and Field 2) and the other field in UKM Paddy field (Field 3), for the year 2014. The soil samples were air dried and ground to pass through a 63-μm sieve. The wet digestion method was adopted to extract heavy metals from soil[19]. One gram of each sample was weighed into a conical flask, then 15 mL of HNO_3 was added followed by 5 mL of $HClO_4$ (3:1) and the left for 2-3 hr in a sand bath. The digested samples were filtered through 0.45 μm pore size Millipore filter paper and made up to 50 mL with deionized water before the metal contents were determined with ICP-Mass spectrometer model EIAN 9000. Soil pH was determined in soil, water ratio of (1:2.5)[20] method whereas total organic carbon[21].

Table 1: Mean values of soil pH, organic matter (O.M.), Cr concentration and soil texture for different sites

Soil properties	Ranau/field 1	Ranau/field 2	Control/field 3
pH	5.28 ± 0.11	5.75 ± 0.16	4.25 ± 0.08
O.M.	10.89 ± 0.16	6.48 ± 0.06	2.91 ± 0.03
Cr	4161.82 ± 472.02	312.51 ± 23.62	35.20 ± 1.36
Soil texture	Silty clay	Clay loam	Clay loam

Note: Means within the same row followed by the same letter are not significantly different to each other at $p > 0.05$

Chlorophyll extracting

Chlorophyll (a, b, and total) analysis pigment contents of plants were extracted by using the formula of Arnon[22]. The leaves were chopped into small pieces that were extracted with 80% acetone. The absorbance was measured at 645 nm and 663 nm for Chl. a, b by using spectrophotometer. Then chlorophyll a, b were calculated according to[23].

Chl. a (mg g^{-1} leaf fresh weight) = [12.7 (OD663) - 2.69 (OD645)] × V/1000 × W

Chl. b (mg g^{-1} leaf fresh weight) = [22.9 (OD645) - 4.68 (OD663)] × V/1000 × W

Where

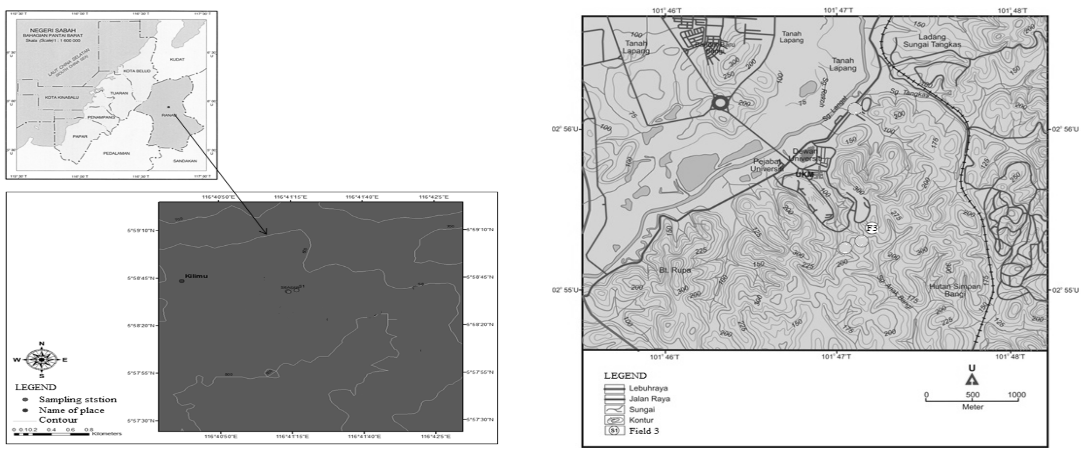
OD = Optical Density

V = Volume of sample

W = Weight of sample

Statistical analysis

The experiment was carried out in triplicate. Statistical analysis of the data was performed by Two-way ANOVA using SPSS software (SPSS ver.20). P values less than 0.05 were considered to be statistically significant. Values were expressed in means ± SD[24].



Study area

RESULTS AND DISCUSSION

Chromium concentration in leaf

Data indicated that significant ($p < 0.05$) differences for Chromium concentration in leaf of paddy in the sites and age of plant (Table 2). Ranau field 1 was superiority in the concentration of chromium metal in leaf of paddy (31.05) mg Kg^{-1}, while the UKM field 3 recorded the lowest mean of Chromium concentrations (2.68) mg Kg^{-1}. The data of plant age showed significant ($p < 0.05$) differences, the concentration of chromium metal was increased with increasing age of paddy (0.23, 6.08, 24.49, 22.09, 22.97) for ages of paddy (17, 47, 77, 107, 145) days respectively, the highest value was recorded at age 77 days (24.49 mg Kg^{-1} but did not differ significantly with age (107 and 145) days. Sites and age of paddy interaction showed significant ($p < 0.05$) differences also, the highest concentration of this metal in the paddy leaf was at the Ranau field (1) and age 77 days that was gave (51.48) mg Kg^{-1}, while the lowest concentrations of this metal was at the UKM field (3) (0.00) mg Kg^{-1}.

Table 2: Effect of sites and age of paddy plant on leaf chromium concentration (mg Kg^{-1})

Sites	17	47	77	107	145	Sites mean
Ranau/Field 1	0.56 ± 0.39	12.92 ± 1.89	51.48 ± 8.66	41.08 ± 10.30	49.22 ± 3.76	31.05 a ± 21.90
Ranau/Field 2	0.12 ± 0.10	3.15 ± 1.94	18.28 ± 4.68	20.21 ± 1.59	17.13 ± 4.25	11.78 b ± 9.06
Control/Field 3	0.00 ± 0.00	2.17 ± 1.41	3.71 ± 0.50	4.97 ± 2.23	2.55 ± 1.12	2.68 c ± 2.04
Age of plant mean	0.23 c ± 0.32	6.08 b ± 5.37	24.49 a ± 21.77	22.09 a ± 16.58	22.97 a ± 20.88	

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at $p > 0.05$

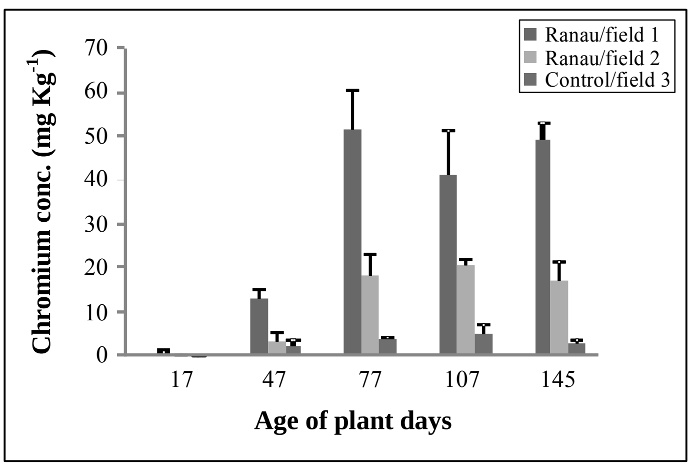


Fig. 1

Chlorophyll a content

Chlorophyll a content in this study decreased significantly with increasing chromium concentration in leaf of paddy in the experiment sites that were studied (Table 3) the order of Chlorophyll a content in leaf of paddy was (2.23 > 2.15 > 1.79) mg g^{-1} FW, for the sites Ranau field 2, control field 3 and Ranau field 1, respectively. Chlorophyll a was record significant negative correlated with a chromium concentration in leaf of paddy was (-0.88). The increasing of age of paddy plant was caused significant decreasing with Chlorophyll a content depends on the amount of chromium that uptake by paddy plant, the order of chlorophyll a content was (2.31, 2.22, 2.05, 2.03, 1.67) mg g^{-1} FW, for ages (17, 47, 77, 107, 145) days, respectively. This is illustrated the value of the correlation coefficient with the accumulated chromium concentration in the leaves of the plant (-0.50). The interaction between the sites and age of Paddy plant showed significant ($p < 0.05$) variation, the highest content of chlorophyll a was noticed in Ranau field 2 and age 107 days, that was gave (2.35) mg g^{-1} FW, but this content did not differ significantly with chlorophyll a content at age (77) days and the same sites, while the lowest chlorophyll a was recorded at Ranau field 1 and age 145 days (1.42) mg g^{-1} FW.

Table 3: Effect of sites and age of paddy plant on chlorophyll a (mg g^{-1} FW) and correlation with leaf chromium

Sites/Ages	17	47	77	107	145	Sites mean	Correlation with leaf chromium
Ranau/Field 1	2.32 ± 0.05	2.11 ± 0.10	1.60 ± 0.00	1.48 ± 0.07	1.42 ± 0.04	1.79 c ± 0.38	– 0.88
Ranau/Field 2	2.31 ± 0.12	2.33 ± 0.10	2.35 ± 0.31	2.35 ± 0.04	1.83 ± 0.06	2.23 a ± 0.25	
Control/Field 3	2.31 ± 0.05	2.22 ± 0.01	2.20 ± 0.19	2.25 ± 0.06	1.75 ± 0.10	2.15 b ± 0.22	
Age of plant mean	2.31 a ± 0.07	2.22 a ± 0.19	2.05 b ± 0.39	2.03 b ± 0.42	1.67 c ± 0.20		
Correlation with leaf chromium	– 0.50						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at p > 0.05

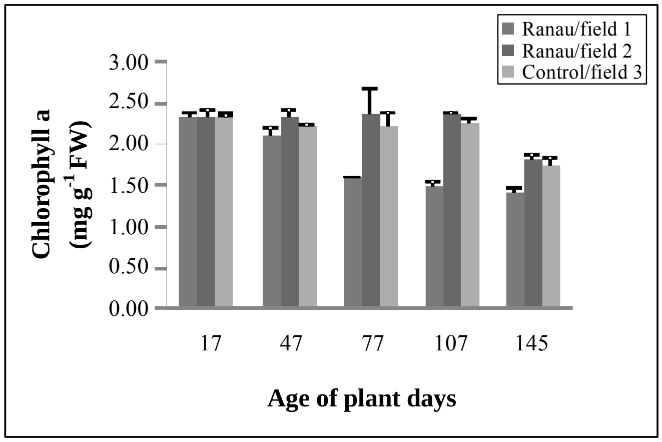


Fig. 2

Chlorophyll b content

Chlorophyll b content data (Table 4) indicated that significant differences effects with using deferent sites, the highest mean of chlorophyll b was at Ranau field 2 (0.84) mg g^{-1} FW, did not significant differ with UKM experimental plot, while the lowest mean was at Ranau field 1 that polluted with chromium metal (0.52) mg g^{-1} FW, as the value of the correlation with the chromium concentration in the plant leaves (-0.95).

Table 4: Effect of sites and age of paddy plant on chlorophyll b (mg g^{-1} FW) and correlation with leaf chromium

Sites/Ages	17	47	77	107	145	Sites mean	Correlation with leaf chromium
Ranau/Field 1	0.84 ± 0.04	0.62 ± 0.04	0.43 ± 0.01	0.39 ± 0.02	0.31 ± 0.01	0.52 b ± 0.20	-0.95
Ranau/Field 2	0.86 ± 0.03	0.85 ± 0.02	0.86 ± 0.06	0.89 ± 0.03	0.72 ± 0.06	0.84 a ± 0.07	
Control/Field 3	0.93 ± 0.08	0.85 ± 0.05	0.83 ± 0.02	0.89 ± 0.02	0.71 ± 0.01	0.84 a ± 0.08	
Age of plant mean	0.88 a ± 0.06	0.77 b ± 0.12	0.71 c ± 0.21	0.73 c ± 0.26	0.58 d ± 0.20		
Correlation with leaf chromium	-0.82						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at $p > 0.05$

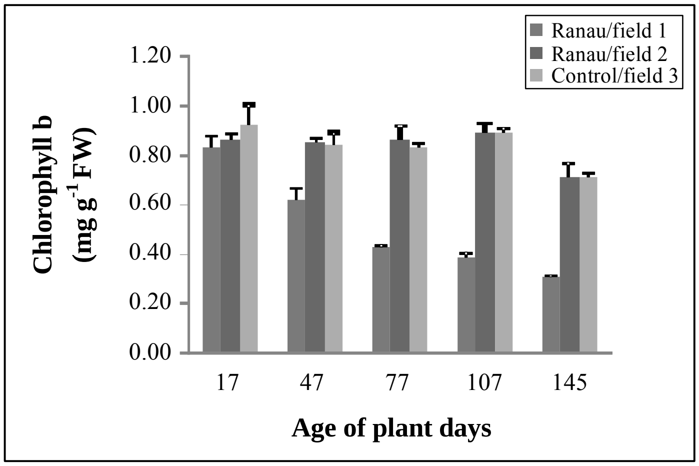


Fig. 3

Increasing of paddy age decreasing significantly chlorophyll b (0.88, 0.77, 0.71, 0.73, 0.58) mg g^{-1} FW for ages (17, 47, 77, 107, 145) days respectively, as the value of the correlation coefficient with chromium concentration (-0.82). The interaction between sites and age of Paddy plant recorded significant variation ($p < 0.05$), the highest content of chlorophyll b was at the UKM experimental plot and age 17 days (0.93) mg g^{-1} FW, while the lowest chlorophyll b was recorded at Ranau field 1 and age 145 days (0.31) mg g^{-1} FW.

Chlorophyll (a+b) Content

Chlorophyll (a+b) content in paddy leaf decreased significantly with increasing chromium concentration in the experiment sites (Table 5) the order of total Chlorophyll (a+b) content in leaf of paddy was (3.07 > 2.99 > 2.31) mg g^{-1} FW, for the sites Ranau field 2, UKM control field 3and Ranau field 1, respectively, chlorophyll (a+b) content in Ranau field 2 did not significant differ with UKM experimental plot, total Chlorophyll (a+b) was record significant negative correlated with a chromium concentration in leaves of paddy was (-0.92). The increasing of age of paddy plant was a decreasing with trend in total chlorophyll (a+b) content depends on the amount of chromium in the leaf of paddy, the order of total chlorophyll (a+b) content was (3.19, 2.10, 2.76, 2.75, 2.25) mg g^{-1} FW, for ages (17, 47, 77, 107, 145) days, respectively. The correlation between total Chlorophyll (a+b) and chromium concentration was (-0.23). Sites and age of Paddy plant interaction showed significant ($p < 0.05$) variation, the highest content of total chlorophyll (a+b) was at the Ranau field 2 and age 107 days, that was gave (3.25) mg g^{-1} FW, while the lowest content of it was recorded at Ranau field 1 and age 145 days (1.73) mg g^{-1} FW.

Table 5: Effect of sites and age of paddy plant on chlorophyll (a+b) (mg g^{-1} FW) and correlation with leaf chromium

Sites/Ages	17	47	77	107	145	Sites mean	Correlation with leaf chromium
Ranau/Field 1	3.16 ± 0.08	2.73 ± 0.11	2.03 ± 0.01	1.87 ± 0.06	1.73 ± 0.03	2.31 b ± 0.57	-0.92
Ranau/Field 2	3.17 ± 0.15	3.18 ± 0.90	3.21 ± 0.25	3.25 ± 0.07	2.54 ± 0.09	3.07 a ± 0.30	
Control/Field 3	3.24 ± 0.07	3.07 ± 0.06	3.03 ± 0.19	3.14 ± 0.05	2.46 ± 0.11	2.99 a ± 0.30	
Age of plant mean	3.19 a ± 0.97	2.10 b ± 0.22	2.76 c ± 0.57	2.75 c ± 0.67	2.25 d ± 0.39		
Correlation with leaf chromium	– 0.23						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at $p > 0.05$

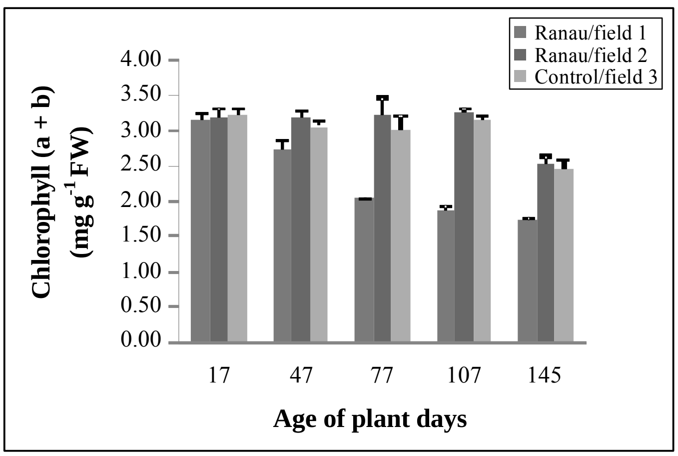


Fig. 4

Chlorophyll (a/b)

Chlorophyll (a/b) ratio values (Table 6) showed significant differences with different locations that were studied, the highest ratio was obtained from Ranau field 1 when the plants were exposed to high concentration of chromium treatment (3.65) as compared to control UKM experiment field 3 (2.55), increasing of chromium concentration significantly increasing Chlorophyll (a/b) ratio, this is confirmed by the value of the positive correlation coefficient with the chromium concentration in the leaves of the plant (0.98). Increasing of age of paddy caused significant increasing with Chlorophyll (a/b) ratio, the correlation between Chlorophyll (a/b) ratio and chromium concentration in the leaves of the plant (0.86), the order of ratio was (2.65, 2.93, 3.03, 2.99, 3.18) for ages (17, 47, 77, 107, 145) days, respectively. The data analysis showed that the Chlorophyll (a/b) ratio was significantly influenced by interaction between sites and age of paddy plant, The highest value was noted at Ranau field 1 and age 145 days (4.53), while the lowest chlorophyll (a/b) ratio was recorded at UKM experimental plot and the same age of plant (2.45).

This experiment has shown the plants that was grown in the sites with the highest levels of Chromium presence showed the greatest effect in terms of Chlorophyll a, b and total Chlorophyll (a+b) and also in the chlorophyll (a/b) ratio. The increase in Chromium presence in the vegetative growth led to a steady decline in Chlorophyll A, B and total Chlorophyll content. At the same time the ratio of Chlorophyll A to B was slightly elevated. The highest ratio (A to B) was seen in the Ranau site, which has the highest levels of Chromium present and the lowest ratio (A to B) was at the UKM (experimental control) site, This was possibly due to Chlorophyll a either collapsing faster or synthesising at a decreased rate when compared with Chlorophyll B, although levels of this also lowered[5]. The relative level of Chlorophyll is often used as an indicator in vegetative growth when studying environmental stress factors, as differences in colour are an obvious indicator of plant health or otherwise and also of the efficacy of photosynthesis[25]. Experimenters have determined that chlorophyll levels are lower in a variety of plant species that have been subjected to heavy metal presence, particularly in Oryza sativa (Rice) in its most mature growth stages, which is the most effective plant stage at which to draw conclusive results[26]. The growth of an organism is severely undermined by the action of heavy metals, which have an inhibitory effect on the plant's enzymes. Enzymes responsible for the synthesis of Chlorophyll may be inhibited by the presence of heavy metals, in this case Chromium. Increased ratios of Chlorophyll (a/b) have also been observed in other plant species e.g. *Spinacea oleracea* (Spinach)[27]. This increased ratio has also been proven to show a change in the PSII – PSI ratio of foliage under stress, and one of the first reactions of vegetation under any sort of stress is the production of free radicals. Free radical production, along with reactive oxygenation, is fomented when vegetation is exposed to heavy metal contaminants and because of the disruption this causes to growth due to oxidation of the cells, the free radicals cause severe harm to molecular structure, including membranes and pigmentation. And is reasoned to be due to the absolute inhibition of the synthetic process providing Chlorophyll pigmentation. Other scientists have also noted a lowering of Chlorophyll levels corresponding to increased levels of heavy metal presence. Chromium has the ability to negatively impact a particularly critical enzyme in the synthesis of Chlorophyll, 'aminolevulinic acid dehydrates' which subsequently impacts on 'aminolevulinic acid' (ALA) itself, resulting in a concentration of ALA arising and a consequent decrease in Chlorophyll levels[28]. The reduction in Chlorophyll A, B and total Chlorophyll in vegetative matter faced with increasing Chromium levels is in fact quite similar to the impact of other environmental contaminants. Lower levels of Chlorophyll could also be the result of electron inhibition during the process of photosynthesis[29]. The negative impacts witnessed as a result of excessive heavy metal contamination in vegetative matter could be due to interference with Chlorophyll creation in one of two ways; possibly by disruption of enzyme function or else as a result of it causing a ferrous shortfall[30].

This particular current study re-affirmed that an increase in Chromium levels led to a reduction in the total Chlorophyll level in the leaves of the subject plant and that the largest decrease in total Chlorophyll was in those plants submitted to the highest levels of Chromium in Ranau sites. Photosynthetic activity had been decreased as a result of raising Chromium levels in the soil which proved the negative impacts of Chromium on the plant's synthesis of Chlorophyll.

Table 6: Effect of sites and age of paddy plant on chlorophyll (a/b) (mg g^{-1} FW) and correlation with leaf chromium

Sites/Ages	17	47	77	107	145	Sites mean	Correlation with leaf chromium
Ranau/Field 1	2.78 ± 0.08	3.42 ± 0.29	3.69 ± 0.46	3.80 ± 0.44	4.53 ± 0.17	3.65 a ± 0.62	0.98
Ranau/Field 2	2.69 ± 0.08	2.75 ± 0.17	2.75 ± 0.56	2.64 ± 0.05	2.56 ± 0.19	2.68 b ± 0.25	
Control/Field 3	2.51 ± 0.25	2.63 ± 0.13	2.65 ± 0.24	2.52 ± 0.09	2.45 ± 0.10	2.55 b ± 0.17	
Age of plant mean	2.65 c ± 0.18	2.93 b ± 0.41	3.03 ab ± 0.58	2.99 ab ± 0.64	3.18 a ± 1.03		
Correlation with leaf chromium	0.86						

Note: Means within the sites column, age of plant row followed by the same letter are not significantly different to each other at $p > 0.05$

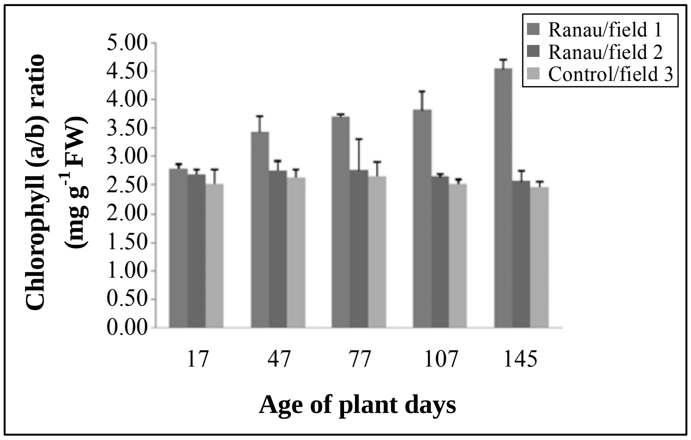


Fig. 5

This relationship between increased Chromium and depleted Chlorophyll has been documented many times; our results are consistent to that of earlier reports by[31-35].

CONCLUSION

Results of this experiment showed that, all traits that s were studied such as chlorophyll a, chlorophyll b, total chlorophyll (a+b) and chlorophyll (a/b) ratio, were affected by the chromium concentration in the leaves of paddy depends on the age of plants, the strongest effect of the high concentration of chromium on decrease the concentration of chlorophyll a, b and (a+b), as well as increased the chlorophyll (a/b) ratio in the leaves of paddy that's grown in these sites.

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