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Eco-Physiological Studies Of Red Mud Waste Discharged From Alumina Industry On a Crop Plant Under Laboratory Controlled Conditions And Its Toxicological Significance.

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Highlights:

- The discharged red mud waste of Alumina industry discharged into Red Mud Pond is dangerously toxic. Lechate leaking from the pond flows as a small canal and joins the river and contaminates the whole water body of Koraput district.
- The lechate contaminates the ground water of the contaminated site, neighboring crop fields and ponds.
- The leached chemicals of red mud pond significantly affected the crop plants at sub-lethal concentrations. The intensity of impact increased with the increase in RMWE concentration.
- Seed germination and seedling establishment was severely affected.
- The leached chemicals / RMWE significantly affected the pigment contents of the seedlings and crop plant.
- RMWE influenced the physiological and biochemical activities of the crop plant thereby reducing the productivity of the crop plant.

Abstract

The main objective of the study was to study the impact red mud waste lechate on a crop plant and to explore the possibility of replacing the rice crop by any other suitable crop. The red mud waste was brought to the laboratory, sun dried, powdered and sieved. The dried powder was mixed with distilled water, stirred for 30days and the supernatant was decanted and kept in glass containers as red mud waste extract (RMWE). This RMWE was used for experiments. The RMWE significantly affected the green gram seed germination and seedling establishment in pot cultures. The pigments were drastically affected in RMWE exposure. The photosynthetic rate and respiration rate significantly depleted in exposed seedlings and green gram plants due to RMWE exposure in pot culture affecting the crop gross production. Depletion of DNA content was also noted in RMWE exposed seedlings and plants indicating the acute toxic nature of leached chemicals leaking from the red mud pond of the industry. Proper dilution of the leached chemicals and careful physical and chemical treatment followed by biological treatment is essential for protection of the crop plants, economically important plants, crop fields, environmental segments and ultimately protection of nature

Keywords: Alumina industry, Red mud waste, lechate, crop plant, seed biology, Physiological activity, Productivity.

Introduction

The TALCO Company was the leading producer of aluminium in central Asia and releases huge amount of red mud waste which was stored in a red mud pond which was not taken care of and the red mud pond collapsed leading to the greatest disaster. NALCO (National Aluminium Co. Limited at Damonjodi, Koraput, Odisha) is the leading industry of World standard for producing Aumina and India became self sufficient in Aluminium production and requirement. Alumina industry is the main centre of air, water and soil pollution. The raw material is processed in the industry to get the finished products which are sold in the market. The industry during processing of the raw materials uses many chemicals, alters physical conditions at different steps to purify the product. In the whole sequences of getting pure alumina, the industry releases many of the chemicals as gases or liquids or solids at different stages of manufacture. The gases are generally released to air through chimneys which causes air pollution. To save the local people from air pollution the height of the chimney is raised to a great height, so that the released polluted air moves to distances. The liquid waste is very thick (red mud=RM) called as slurry is discharged into a stocking area (RM Pond) for air drying the red mud waste. The red mud waste is stored in the red mud pond which is surrounded by natural hills from all sides and a small link is left out which is closed by earthern dam in the form of a dam. Earlier no body probably thought of leakage and leaching. The pond is not plastic lined or cemented. Continuous leaching takes place from red mud pond and the surrounding land mass is contaminated by the leached chemicals. After many years, now it was observed that small line steams are oozing from the red mud pond and flows downwards and joins small streams and finally these streams join and reach to a small river contaminating the aquatic system. It was also observed that the lechate flow towards crop fields located nearby. These lechate accumulate in the crop fields and keep the field wet. Generally people of the area cultivate their staple food rice. These lechate are light brown in color. It was observed that the rice crop absorbs these lechate and the color of the leaf, root and leaf petiole slowly turn brown. This color also passes into the grain/seed. The rice grain also looked brown. The rice once cooked, the cooked rice also turns light brown or light yellow and has a very bad smell. This rice grain was neither fit for consumption or for sale. Hence the farmers of those areas abandoned the crop fields, as was learnt by personal interaction with the farmers. The farmers complained before the industry people regarding crop loss, browning of rice grain and their loss. The industry people partially paid some amounts through rehabilitation package which was not enough for life maintenance and to support family. So these farmers changed their profession instead of cultivation, they worked as laborers in the industry. Keeping in view; the discharge of red mud effluents of the industry into red mud pond and leaking of chemicals from the red mud waste dumping pond of the industry entering into water bodies and during rainy season entry of these chemicals and lechate along with runoff water and over flow of runoff water of the paddy fields, their entry into fresh water bodies like fish ponds, canals, rivers and the water reservoirThe local report from the local residents about loss of rice crop & browning of rice dragged our attention and hence, this project was planned to evaluate the eco-toxicological effects of the leached waste of the Red Mud Pond (RMP) of Alumina industry / RMWE prepared on the seed biology, growth and physiological activity of a green gram crop plant under laboratory controlled conditions.

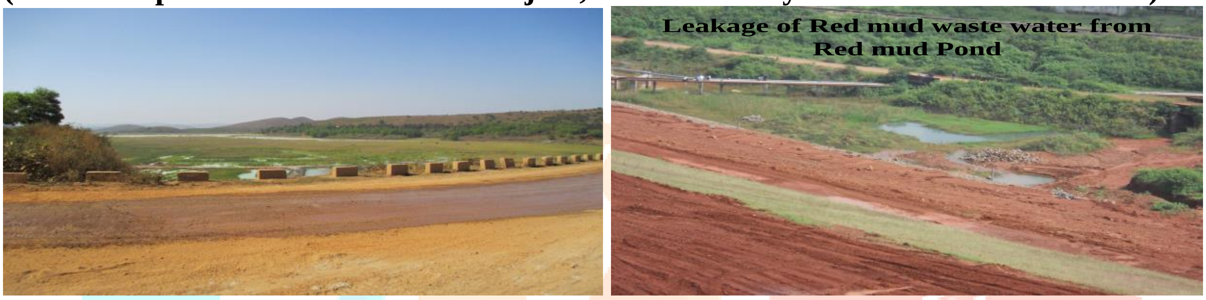
Material & Methods:

Location of the industry:

This industry is the largest integrated Bauxite Alumina-Aluminium complex in India. NALCO produces calcined allumina at refinery complex, Damonjodi. The mines and refinery complex of NALCO, Damonjodi is situated at Similiguda block, under Potangi tahasil in the district of Koraput, Odisha state, India. The industry is located at latitude 18^0-6'--18^0-58' towards North and longitude 82^0.57'-83^0.04' East.

(Satellite Map of Damonjodi area showing Alumina industry and Photo of the Industry)

(Red mud pond of NALCO at Damonjodi; Man made Dyke in one side of the RMP)



(Photo of lechate flowing as a small stream from Dyke and Lechate leaking from the RMP of the industry at Damonjodi, Koraput)

Toxicant: Red mud waste extract:

The red mud collected from RM pond is brought to the laboratory. The slurry was air dried, powdered and sieved. Red mud waste powder was kept in plastic containers for laboratory experimental study. A known (2kg.) quantity of red mud dried powder is mixed with known amount (2lit.) of distilled water in 5liter glass jar and stirred for 15 days with the help of Remi stirrer with medium speed for one hour and allowed to rest for 2hours. The same process was continued for the whole day. It was allowed to settle over night. The process of stirring was repeated for 15days. After 15days the supernatant was decanted and filtered through a Tea strainer (plastic filter) to remove visible suspended particles. It was kept inside a refrigerator for future use. On the day of use the supernatant extract was again passed through a multi-sieve soaking and filtering system and the obtained extract is the extracted leached chemical RMWE) of the waste, which is used for e experiments.

Test system: Green gram seeds (Eng. Mung, Green gram; Odia: Muga)

Botanical name of the crop plant: *Vighna radiata*,(L.) Wilczek

Pure line uncontaminated seeds of green gram (Odia: Muga) were obtained from Pulses and Millet Research Station, OUAT, Ratanpur, Ganjam.

From each pot shoot and root lengths of the seedlings were measured in replicates with the help of a scale and expressed in cms. The fresh and dry weight of seedlings, roots and shoots was taken separately by a single pan electric balance. The amount of total chlorophyll and total phaeophytin was calculated by using the formula given by Vernon (1960). The amount of carotenoid was calculated by using the formula given by Davies (1976). The evolution of oxygen due to photosynthesis and consumption of oxygen and release of carbon dioxide in respiration were measured manometrically with the help of a Photo-Warburg's apparatus (New Paul, India) following the procedure of Hannan and Patouillet (1972) and Oser (1965). Total DNA was measured following the procedure of Herbert *et al*., (1971) by diphenylamine reaction method. All the obtained values were statistically analysed.

Results:

Analysis of mud of red mud pond (Patnaik *et al.*, 2018a)**:**

Analysis of red mud waste revealed that the waste is a mixture of the following chemicals expressed in percentages: Al_2O_3 =19.11%; Fe_2O_3= 56.88%; TiO_2= 4.56%; SiO_2= 5.97%; Na_2O = 3.86%; pH = 9.90; Return water pH = 14; NaOH =30-35% and Na_2CO_3 = 65-70%. The physical and chemical properties of the waste were as follows:

In pot culture, the deduced lethal concentration values for green gram seeds were LC_0=0.075 ml/liter (v/v); LC_{05} =0.2ml/liter; LC_{10} =1.5ml/liter; LC_{50} =3.0ml/liter; LC_{90} =5.0 ml/liter and LC_{100} =5.6 ml/liter when exposed to lechate (RMWE) of the red mud waste. The percent of seed germination increased with the increase in time and decreased with the increase in toxicant concentration in pot culture (Fig.1). It was observed that the impact of lechate was less in pot culture compared to petriplate culture, where the seed and seedlings come in direct contact with the lechate chemical. In pot culture the lechate chemical gets mixed with the soil mixture and the toxicity reduced.

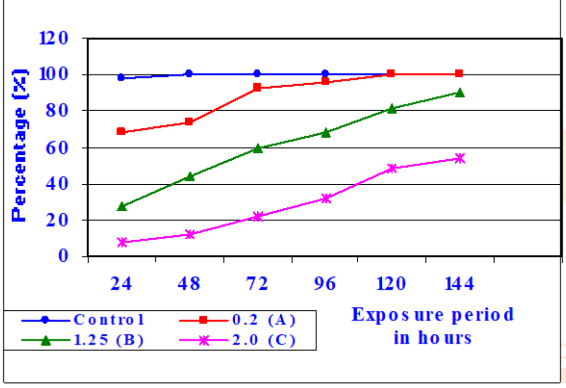


Fig. 1. Changes in seed germination & seedling establishment of green gram seeds at different concentrations red mud waste extract.

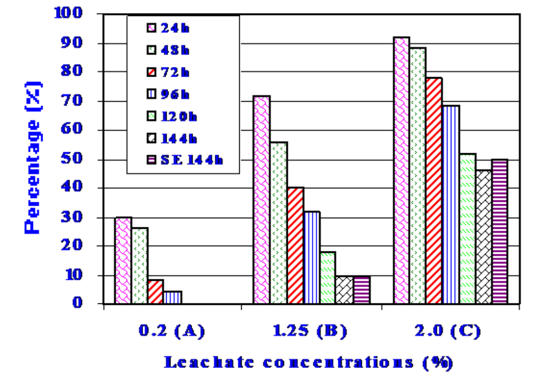


Fig.2. Percent inhibition in seed germination in Green gram seeds exposed to different leachate concentrations in pot culture.

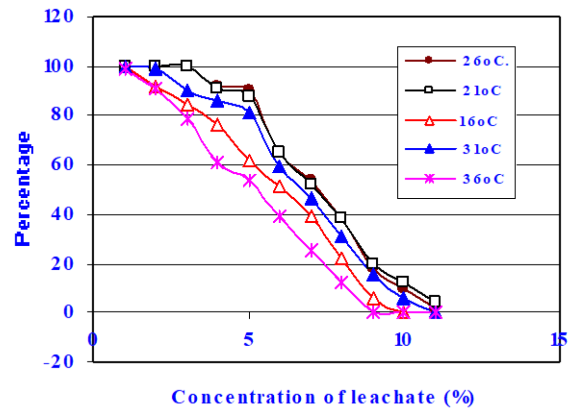


Fig. 3: Effect of temperature stress on Green gram seed germination in pot culture.

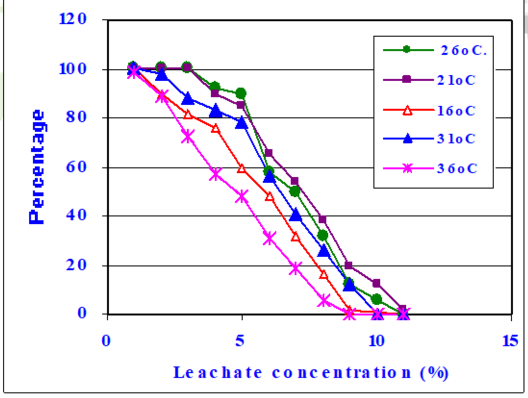


Fig. 4: Effect of temperature on seedling establishment of germinated seedlings in pot culture.

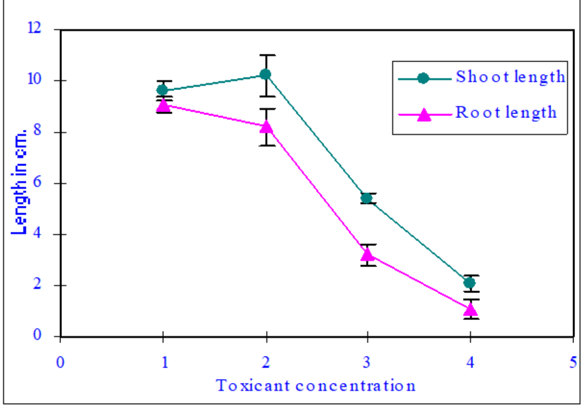


Fig. 5: Showing changes in Shoot, Root length of 144 hours old green gram seedlings in pot culture.

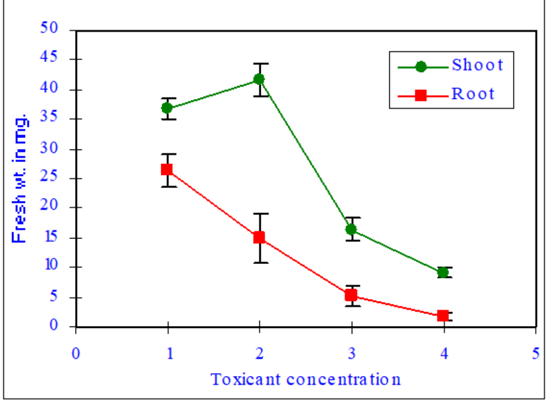


Fig.6: Showing changes in Shoot and Root fresh wt of 144 hours old green gram seedlings in pot culture.

However, in case of pot culture experiments, we could not find such a trend. Decrease in percentage of green gram seed germination was noted at higher concentrations of lechate of red mud waste and seedling growth followed the same behavior in lechate exposed seedlings (fig.1-2). The irregular growth and establishment of seedlings was noted and no regular sequential trend was observed. Interestingly at in and around LC_{50} concentrations of lechate some seedlings grew well even better than the control seedlings. It is not possible to predict the real cause at this stage. The experiment was repeated several times to ascertain the above observations. But to our surprise, never we got the same type of result to reach to a firm conclusion. From the toxicity test, it was found that all the seeds germinated (100%) up to 0.2% of the lechate. With the increase in lechate concentration, the percent of seed germination decreased, showing a significant negative correlation. 90% seed germination was recorded up to 1.25% lechate concentration. At 2.0% lechate concentration, 50% seed germination was noted. At 2.6% lechate concentration, only 10% of exposed seeds germinated, when compared to control seeds. Hundred percent seed germination was recorded in the control set, where all the seeds germinated. Whereas, in the exposed sets the percent of seed germination decreased significantly when compared to the control set seeds. With the increase in exposure period (in hours), the percent of seed germination increased showing a positive correlation and with toxicant concentration, it showed a negative correlation. Hundred percent seedlings established in the control set and at 0.1 and 0.2% red mud waste lechate concentration. With the increase in lechate concentration, the percentage of seedling establishment decreased showing a negative correlation ($r=0.955$; $p\leq 0.01$). 100% establishment was noted at 0.2% lechate concentration; 90% seedling establishment was recorded in 1.25% lechate concentration and 50% seedling establishment was recorded at 2.0% lechate concentration. No seedling establishment was noted beyond 2.8% lechate concentration of red mud waste collected from red mud pond. From the data, it can be inferred that 2.8% of red mud lechate concentration is deadly toxic for the green gram seeds and seedlings. Control seeds and seedlings looked healthy and showed better growth when compared to exposed seeds and seedlings during the whole period of experimentation. After toxicity testing, experiments were conducted to test the impact of 5°C and 10°C higher and 5°C and 10°C lower when compared to the normal temperature of 26°C at the study site. Study site temperature was maintained in the laboratory for the experiments. This temperature stress experiment was conducted for the first time in our laboratory under controlled conditions in a seed germinator (Remi). When the normal temperature of 26°C was lowered by 5°C to 21°C, no significant variation was noted in percentage of seed germination. Little variation like little decrease at lower concentration and little increase at higher concentration of lechate was observed. But when the temperature was lowered by 10°C, when compared to normal temperature, significant variation was noted. At 16°C, the percentage of seed germination decreased significantly showing a significant negative correlation ($r= 0.971$; $p\leq 0.01$) indicating the impact of temperature stress (Fig.3). The mean experimental temperature of 26°C was raised by 5°C in the seed germinator and it was found that the impact was notable. With the increase in lechate concentration, the percentage of seed germination decreased from 100% to 90% at 0.2% lechate; decreased to 81% at 1.25% lechate and depleted to 46% at 2% lechate. No germination was found beyond 2.8% lechate concentration (Fig.3). When the temperature was raised by 10°C above the normal temperature, the percentage of seed germination significantly decreased from 100% in control to 79% at 0.2% lechate concentration. With the increase in lechate concentration to 1.25%; the percentage of seed germination decreased to 54% at 1.25% lechate and further depleted to 25% at 2% lechate. No germination was found beyond 2.4% lechate concentration (Fig.3). When the normal temperature of 26°C was lowered by 5°C to 21°C, no significant variation was noted in percentage of seedling establishment (Fig.4). Insignificant variation like little decrease at lower concentration and little increase at higher concentration of lechate was observed. But when the temperature was lowered by 10°C, when compared to normal temperature, significant variation in seedling establishment was noted. At 16°C, the percentage of seedling establishment decreased significantly showing a significant negative correlation ($r= 0.979$; $p\leq 0.01$) indicating the impact of temperature stress. The seedling establishment decreased from 100% to 82% at 0.2% lechate concentration and the value further depleted to 60% at 1.2% lechate concentration and significant depletion was noted at 2% lechate, where the percentage seedling establishment decreased to 32% only and no germination was recorded beyond 2.6% lechate concentration. The mean experimental temperature of 26°C was raised by 5°C in the seed germinator and it was found that the impact was notable. With the increase in lechate concentration, the percentage of seedling establishment decreased from 100% to 88% at 0.2% lechate; decreased to 78% at 1.25% lechate and depleted to 41% at 2% lechate. No seedling establishment was found beyond 2.6% lechate concentration (Fig.4). When the temperature was raised by 10°C above the normal temperature, the percentage of seedling establishment significantly decreased from 100% in control to 73% at 0.2% lechate concentration. With the increase in lechate concentration to 1.25%; the percentage of seedling establishment decreased to 54% at 1.25% lechate and further depleted to 25% at 2% lechate. No germination was found beyond 2.4% lechate concentration (Fig.4) indicating serious impact at higher concentrations of the lechate leaking from red mud pond. It was observed that lower temperature has no significant impact on the seed germination and seedling establishment when compared to higher temperature. The toxicant used in this study is deadly toxic and should be carefully used for the experiments. Significant decrease in germination rate of seeds and seedling establishment indicated the toxic nature of the red mud lechate. The observed results were significant when compared with the control data. The impact study of temperature stress is very important, as in nature the live biota experiences these temperature fluctuations like high temperature in summer and low temperature in winter. It was observed that the root tip of the lechate exposed seedlings turned brown and tends to move upwards i.e. away from the toxic medium, when compared to control seedling roots. The exposed seedling leave tips turned brown and curled. The change in color might be due to the absorption and transportation of the RMW lechate to the exposed seedling leaves. An experiment was conducted to study the effect of the RMW lechate (toxicant) in pot culture. The obtained data can be compared with the data obtained in the seed germinator, when the environmental parameters were controlled. The shoot length of the lechate exposed green gram seedlings declined from 9.6 ± 0.4 to 2.1 ±0.3 cm at conc. C, where, a maximum of 78.12 % decrease was recorded. In pot culture the decrease was 78.12% in shoot length. So here, 7.84% benefit in shoot length was available in pot culture. Pot culture experiments showed better results over petriplate experiment results. The benefit might be due to the interaction of environmental parameters and most important was the availability of nutrients in the soil. And in addition, the advantage of toxicant natural detoxification or binding of the toxicant with the soil might have played a crucial role in giving such advantage in the pot culture. The root length in pot culture declined from 9.1± 0.3 cm to 1.1± 0.4 cm at concentration C. The decrease was linear and steady with the increase in toxicant concentration, where a maximum of 87.91 % decrease was recorded. In pot culture, this was not significant. The non-significant difference observed in the pot culture indicates significantly the adverse effect of the toxicant. The root / shoot length ratio increased from 1.05 to1.91, showing a maximum increase by 81.90% at conc.- C. The shoot fresh weight increased from 36.8 ± 1.8mg to 41.6 ±2.8mg at concentration A and than the fresh weight value decreased to 16.4 ± 1.9mg at conc. B. The shoot fresh weight declined from 36.8 ± 1.8mg to 9.2 ±0.8mg at concentration C, showing a maximum decline by 75%, when compared to control. The decrease in shoot fresh weight was again linear, with the increase in toxicant concentration except at conc.-A. The shoot weight in exposed seedling declined indicating decrease in growth induced by the toxicant. The root fresh weight declined from 26.4 ±2.8mg to 1.8 ±0.6mg at conc-C, where a maximum of 98.18% decrease was recorded, when compared to the control value, in pot culture (Fig.7). The shoot dry weight decreased from 8.6 ± 0.8 mg to 1.6 ±0.3 mg at conc.-C. A maximum of 81.39 % decrease when compared to control was recorded. The root dry weight declined from 6.2 ± 0.6mg to 0.7 ±0.2mg at conc. C. Here, a maximum of 88.71% decrease in root dry weight was recorded at conc.-C, when compared to control value. The root / shoot dry weight ratio value increased from 1.39 to 2.31 at conc. B and ratio value declined to 2.28 at conc. C. A maximum of 66.19% and 64.03 % increase in the ratio value was recorded at conc. B and conc. C, respectively. All the exposed parameters were found to be less than the control values except at few cases, where an increase was recorded. Higher toxicity values were recorded in petriplate cultures and less toxicity values were recorded in pot culture. The difference in both was the environmental influence and the common factor in both the experiment was the application of the toxicant at the same concentration. The ANOVA tests indicated that there was no significant difference between rows. However, significant differences between columns were marked.

Fig.7: Changes in Shoot and Root dry wt. of 144 hours old green gram seedlingss pot culture.

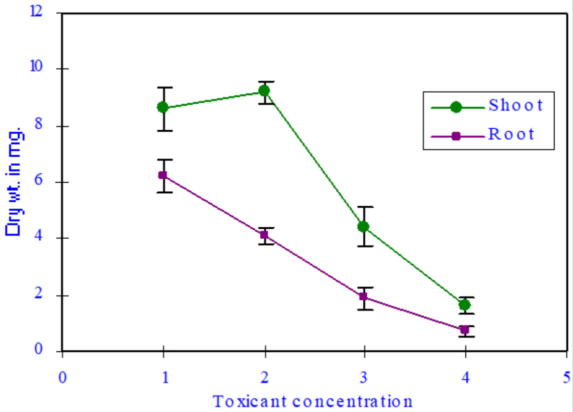


Fig.8.Changes in the total chlorophyll content of leaves of green gram plants in pot culture.

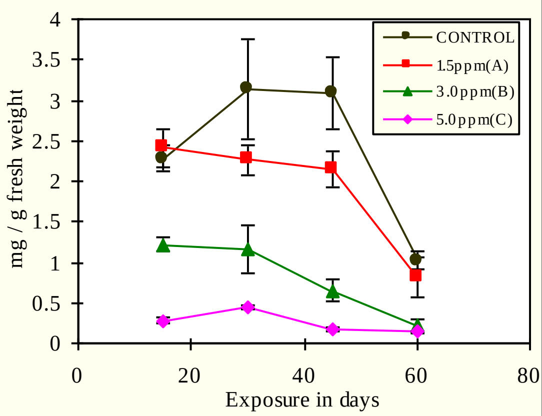


Fig.9: Changes in the total phaeophytin content of the leaves of green gram plants a in pot cultures.

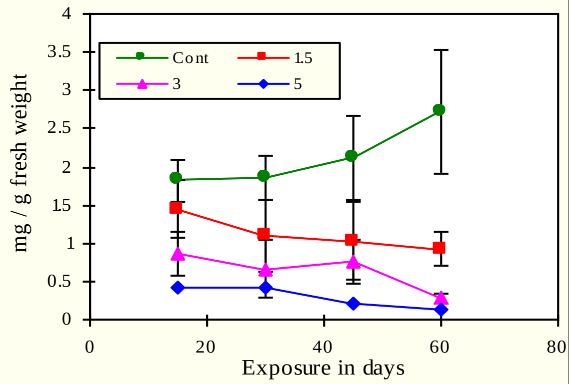
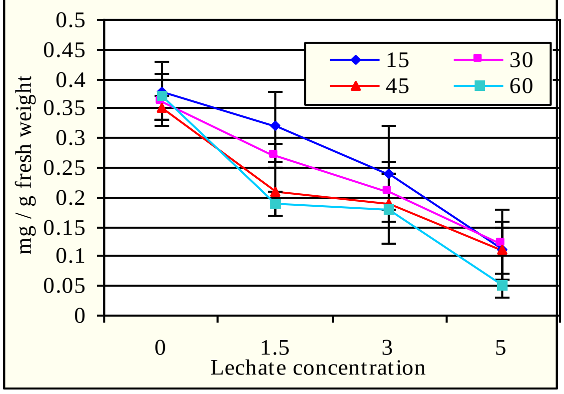


Fig.10: Changes in DNA content in the leaf of control & lechate exposed green gram plants at different exposure periods.



Changes in plant pigment content, pigment ratio values and percent change in pigment content in exposed seedlings when compared to control seedlings in pot culture has been shown in Figures displayed below. Chlorophyll-a decreased from 0.56mg / g to 0.08 mg / g at 5ppm toxicant concentration, Chlorophyll-b decreased from 0.54mg / g to 0.03mg / g at 5ppm toxicant concentration, Chlorophyll-a decreased from 0.56 mg / g to 0.08mg / g at 5ppm toxicant concentration, total chlorophyll decreased from 1.19mg / g to 0.11 mg / g at 5ppm toxicant concentration (Fig.8). Total pheophytin decreased from 0.96mg / g to 0.014mg /g at 5ppm toxicant concentration and total carotene content decreased from 0.0146mg g^{-1} to 0.0011mg g^{-1} fresh weight at 5ppm toxicant concentration (Fig.9). The chlorophyll content, phaeophytin content and carotene content decreased significantly with the increase in RMWE concentration in exposed seedlings when compared to control seedlings in pot culture. The exposed leaves curled and browning of the tips were noticed. The control leaves remained healthy throughout. At very high concentration no shoot was marked but rudimentary root was marked. The changes in total chlorophyll in control and leached chemicals of the red mud waste exposed green gram plant leaves at different period of exposure in pot culture. The total chlorophyll content was 2.28 ± 0.16 mg/g fresh weight on 15th day of exposure in the control green gram plant leaf. The total chlorophyll content increased from 2.28±0.16mg/g fresh weight to 2.44±0.24mg/g fresh weight at conc.-A of the toxicant and on 15th day of exposure in pot culture, where a maximum 7.02 % increase was recorded. With the increase in toxicant concentration, the percent decrease of total chlorophyll increased showing a significant correlation on 30th day of exposure, the total chlorophyll content decreased steadily from 3.14 ±0.62mg/g fresh weight to 0.41±0.02 mg/g fresh weight at conc.-C and where, a maximum of 86.94% decrease was recorded. On 45th day of exposure, the total chlorophyll content declined from 3.08±0.44 mg/g fresh weight to 0.08±0.02 mg/g fresh weight at conc.-C. On 45th day of exposure, a maximum of 97.4% decrease, when compared to control value was recorded at conc.-C. The decrease in values with the increase in toxicant concentration and the increase in percent decrease with the increase in toxicant concentration indicated stress. On 60th day of exposure, the total chlorophyll content decreased from 1.01±0.11mg/g fresh weight to 0.01± 0.005mg/g fresh weight at conc.-C, where, a maximum 99.02 % decrease was recorded. In all the three concentrations of the toxicant, the total chlorophyll content decreased with the increase in exposure period sharing a negative correlation and the total chlorophyll content declined with the increase in toxicant concentration indicating the existence of a negative correlation. The changes in total phaeophytin content in control and leached chemicals of the red mud waste exposed green gram plant leaves at different days of exposure and at different concentrations of the toxicant. The figure indicated the percent change in phaeophytin content in the exposed seedlings in pot culture. The correlation coefficient values of pigment content of the 144 hour old seedlings in petriplate culture, where significant negative correlations were marked between toxicant concentrations and pigments like total chlorophyll, phaeophytin and carotene content. The total phaeophytin content declined from 1.82±0.27 mg/g fresh weight to 1.74±0.38 mg/g fresh weight conc.-A, from 1.82±0.27 mg/g fresh weight to 0.91 ± 0.29 mg/g fresh weight at conc.-B, and from 1.82 ± 0.27 mg/g fresh weight to 0.52 ± 0.18 mg/g fresh weight at conc.-C on 15th day of exposure. With the increase in toxicant concentration, the phaeophytin content decreased and the percent decrease increased with the increase in toxicant concentration and 95.95 % maximum decrease was recorded at concentration C. After 30 days of exposure the total phaeophytin content decreased from 1.86 ± 0.28 mg/g fresh weight to 1.31 ± 0.47 mg/g fresh weight at conc.-A, from 1.86 ± 0.28 mg/g fresh weight to 0.76±0.38 mg/g fresh weight at conc.-B, and from 1.86±0.28 mg/g fresh weight to 0.43 ± 0.28mg/g fresh weight at conc.-C. A maximum of 76.88 % decrease in phaeophytin content was recorded at conc.-C, on

30th day of exposure. The total phaeophytin content decreased from 2.12± 0.54mg/g fresh weight to 1.42±0.51 mg/g fresh weight at conc.-A, to 0.54±0.28 mg/g fresh weight at conc.-B and to 0.28±0.08 mg/g fresh weight at conc.-C on 45th day of exposure. A maximum of 86.79% decrease was recorded at conc. C and on 45th day of exposure. The total phaeophytin content decreased from 2.72±0.82 mg/g fresh weight to 1.14±0.22 mg/g fresh weight at conc.-A, to 0.31±0.07 mg/g fresh weight at conc. B and to 0.11±0.02 mg/g fresh weight at conc. C and was recorded on 60th day of exposure in pot culture. A maximum of 95.95 % decrease was recorded at concentration C and on 60th day of exposure. With the increase in toxicant concentration and day of exposure, the phaeophytin content decreased showing a negative and significant correlation. With the increase in exposure period and toxicant concentration, the percent decrease in phaeophytin content showing the existence of positive correlation. Both the assessments indicated the toxicant stress.

The DNA content of the green gram plant leaves ranged between 0.35±0.02mg/g fresh weights to 0.38±0.05mg/g fresh weight during the entire period of experimentation (60days- at 15d interval) in the control pot set. The DNA content of the lechate exposed green gram plant leaves decreased from 0.38±0.05mg/g fresh weight to 0.32±0.06mg/g fresh weight at conc. A after 15days of exposure.

Fig.11: Changes in DNA content in the root of control and lechate exposed green gram seedlings at different exposure periods.

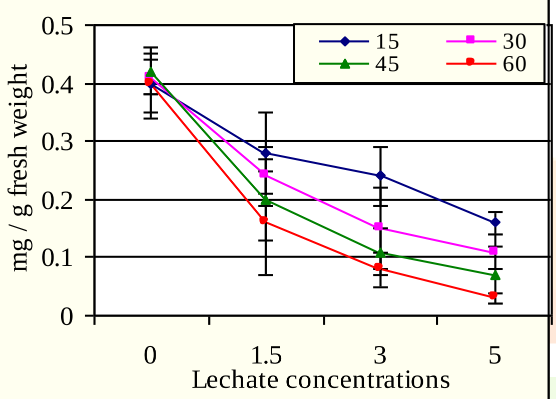
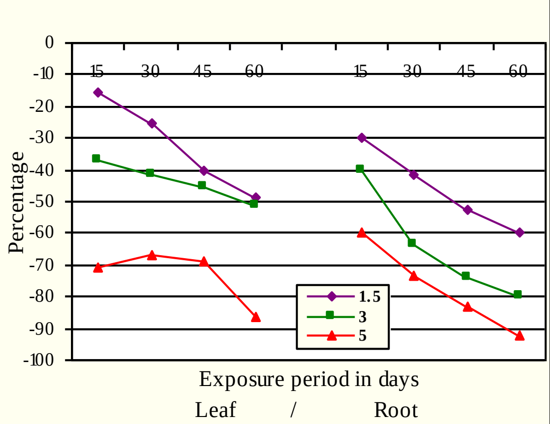


Fig.12: Percent decrease in DNA content in leaf and root of green gram lechate exposed plants in pot culture.



The DNA content of the lechate exposed green gram plant leaves decreased from 0.38±0.05mg/g fresh weight to 0.24±0.08mg/g fresh weight at conc. B after 15days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.38±0.05mg/g fresh weight to 0.11±0.05mg/g fresh weight at conc. C after 15days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.36±0.04mg/g fresh weight to 0.27±0.02mg/g fresh weight at conc. A after 30days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.36±0.04mg/g fresh weight to 0.21±0.03mg/g fresh weight at conc. B after 30days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.36±0.04mg/g fresh weight to 0.12±0.06mg/g fresh weight at conc. C after 30days of exposure in pot culture. The DNA content of the lechate exposed green gram plant leaves decreased from 0.35±0.02mg/g fresh weight to 0.21±0.04mg/g fresh weight at conc. A after 45days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.35±0.02mg/g fresh weight to 0.19±0.07mg/g fresh weight at conc. B after 45days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.35±0.02mg/g fresh weight to 0.11±0.04mg/g fresh weight at conc. C after 45days of exposure in pot culture. The DNA content of the lechate exposed green gram plant leaves decreased from 0.37±0.04mg/g fresh weight to 0.19±0.02mg/g fresh weight at conc. A after 60days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.37±0.04mg/g fresh weight to 0.18±0.06mg/g fresh weight at conc. B after 60days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.37±0.04mg/g fresh weight to 0.05±0.02mg/g fresh weight at conc. C after 60days of exposure in pot culture (Fig.10-11). The DNA content of the lechate exposed green gram plant leaves decreased from 0.4±0.06mg/g fresh weight to 0.16±0.02mg/g fresh weight at conc. C after 15days of exposure (Fig.11). The DNA content of the lechate exposed green gram plant roots decreased from 0.41±0.03mg/g fresh weight to 0.24±0.05mg/g fresh weight at conc. A after 30days of exposure. The DNA content of the lechate exposed green gram plant roots decreased from 0.41±0.03mg/g fresh weight to 0.15±0.07mg/g fresh weight at conc. B after 30days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.41±0.03mg/g fresh weight to 0.11±0.03mg/g fresh weight at conc. C after 30days of exposure in pot culture. The DNA content of the lechate exposed green gram plant leaves decreased from 0.42±0.04mg/g fresh weight to 0.22±0.07mg/g fresh weight at conc. A after 45days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.42±0.04mg/g fresh weight to 0.11±0.04mg/g fresh weight at conc. B after 45days of exposure. The DNA content of the lechate exposed green gram plant leaves decreased from 0.42±0.04mg/g fresh weight to 0.07±0.05mg/g fresh weight at conc. C after 45days of exposure in pot culture. The DNA content decreased by 15.9%, 36.8% and 71.1% at conc. A, conc. B and conc. C after 15days of exposure in the lechate exposed leaves of green gram plants. The DNA content decreased by 25%, 41.7% and 66.7% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed leaves of green gram plants. The DNA content decreased by 30%, 40% and 60% at conc. A, conc. B and conc. C after 15days of exposure in the lechate exposed roots of green gram plants. The DNA content decreased by 41.5%, 63.4% and 73.2% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed roots of green gram plants. The DNA content decreased by 52.4%, 73.8% and 83.3% at conc. A, conc. B and conc. C after 45days of exposure in the lechate exposed roots of green gram plants. The DNA content decreased by 60%, 80% and 92.5% at conc. A, conc. B and conc. C after 60days of exposure in the lechate exposed roots of green gram plants (Fig. 12). The leaf and root of control green gram plants did not show any residual accumulation of alumina indicating as standard control. The figure indicated clearly that the roots of the exposed green gram crop plants accumulated more amount of alumina compared to the leaves of the green gram crop plants in pot culture and the amount of alumina increased with the increase in exposure period and lechate concentration. The ANOVA test indicated the existence of significant differences between rows and columns.

Discussion

Aluminium poisoning is responsible to induce browning of roots and stem, yellowing of leaves, reduction in root growth by way of decreasing the root length, inducing depletion in growth and it can cause folding or curling of leaves and death of apical tissues (Foy *et al*., 1978, Matsumoto, 2000 and Singh *et al*., 2017). The above authors also indicated that the young leaves are more affected than the old leaves when exposed to aluminium based toxicants. The above observations are the possibilities when the exposed seedlings accumulate significant amount of aluminium. When all the data were considered along with the statistical data it can be inferred that the toxicant was less effective in pot culture experiments compared to petriplate cultures (Patnaik *et al*., 2017, 2018a,b). In pot culture experiments, the environmental factors played a crucial role and reduced the effect of the toxicant on the crop plant. In both the cases the root was more affected than the shoot, in all the variable parameters. The residual accumulation of the toxicants in the exposed rice plants could have provided much better information. But due to lack of facilities and equipments, residual analysis part was not carried out. All the obtained data were statistically significant. Further, higher-level work is necessary to assess the toxic nature and site of the action studies. Chen *et al*. (2013) reported that exposure to higher concentrations of aluminium, leads to higher and significant accumulation aluminium in root and leaf affecting the root and leaf in the seedling stage. It is observed in our studies that the seedlings absorb aluminium through the root which is translocated to leaves via stem. Initially the root is affected and turns brown, than the stem and finally the exposed leaves. During toxicity and germination studies, a careful observation can indicate the movement of the colour waste into the germinating seedlings by change in colour. Chen *et al*. (2013), Singh *et al*., (2017) and Rahman *et al* (2018) reported that red mud waste can generate excess reactive oxygen species, disrupts physiological processes like root elongation, depletion in total biomass, decrease in net productivity, deposition of chemicals like lignin and callose in exposed root tips, interfering in ionic transport, inhibition in photosynthesis and respiration, decrease in all physiological and metabolic activity, disruption Na^{+}-K^{+} pump, Ca^{+2}- pump. Increase in studied parameters at 0.2%, (maximum allowable concentration) lechate concentration over the control value suggests the idea that if the leaked chemicals can be heavily diluted by uncontaminated water than this diluted water can be used for irrigation of crop fields, where green-gram crop is being cultivated. The data showed the percent changes in the aforesaid variables, where at 0.2% lechate concentration, shoot showed better results over the root. The exposed roots were more affected than the shoot. Sahu (1987) and Sharma (2013) clearly opined that the roots are exposed to the toxicant and the first site of toxicant absorption, when compared to shoot. Unlike heavy metals like mercury which were absorbed and transported to the shoot and the leaf, the site where photosynthesis occurs was affected the most. The impact of alumina on the photosynthetic rate can also be serious like mercury and cadmium but the lower concentration values of alumina at times may not be affecting the productivity rate. This alumina at higher concentrations can be as hazardous as mercury and cadmium. The pigment, chlorophyll was not affected at 0.2% red mud waste lechate, whereas the pigments like phaeophytin and carotene were affected significantly at all concentrations. Photosynthetic pigments of the plant system play a vital role in trapping solar energy (Prusti and Panigrahi, 2017). The pigments are known to participate in generation of energy for CO_2 fixation (Kashyap and Gupta, 1981). Zingmark and Miller, (1975), De Phillipis and Pallaghy (1976) and Sahu and Panigrahi (2002) reported that heavy metals inhibit photosynthesis. Rai *et al*, (1981) reported that heavy metals reduce chlorophyll content of exposed cyanobacterium. It can be inferred from the reports of above authors and many more that heavy metals, industrial wastes and leached chemicals from solid waste and liquid waste dumping sites can cause severe impact on all plant and animal life forms. At very low concentrations the chemicals as singular unit or as a complex unit might trigger higher rate of photosynthesis or higher values of chlorophyll content. But this can not be attributable to either stimulatory effect or regulatory effect of the toxicant. It was also observed that at very low residual accumulation such an effect can be marked but with the increase in residual accumulation of the toxic chemicals in the plant body (may be a cyanobacterium or a crop plant or any plant for the purpose- may be helpful or harmful) inhibitory effects were observed. Mingyuan *et al*., (2020) reported that the bauxite mining area should be properly managed to check pollution by bauxite mining soil. The same author reported the adverse impact of bauxite mining soil on growth performance of *Jatropha curcas.* Kovacs-Bokor *et al*., (2018) reported significant effect of industrial sludge-soil mixture on wheat and white mustard by stimulating the germination of seeds and growth of plumule in exposed plants compared to standard control seed germination and plumule growth. Fourrier *et al*., (2021) explained how Raw and Gypsum modified bauxite residues modify and affect seed germination and root development in white mustard, *Sinapis alba* (L.). Chauhan and Ganguly (2011) standardized the rehabilitation protocol using vegetation cover for bauxite wastes (red mud) in eastern India and indicated the advantages of rehabilitation protocol for reducing the impact of rd mud wastes. Misik *et al*., (2014) reported that red mud is dangerously toxic, caused mutagenicity and also caused DNA damage in humans. The same author also indicated that red mud release in to the environment is not a local problem but a global problem. Panda *et al*., (2021) cautioned that restoration of mining overburden disposal area was a challenging effort to minimize environmental impacts and support sustainable agricultural production. Further work is needed to find out possible ways of detoxification of the leached chemicals of the red mud. No doubt, leaching will be there, in addition ground water contamination cannot be avoided, as there is no bottom lining. At least, care could be taken to stop surface leaching leading to run off water, entering into crop fields and water bodies. From the above tables and figures it can be clearly stated that the red mud waste lechate is highly toxic and can severely affect the crop plants in contaminated site. This attempt was made to test whether green gram crop can be a substitute for rice crop in the red mud waste contaminated sites.

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Declarations:

Author Contribution statement

Dr. A. Leelaveni- Experiment planning and execution of the project, field visit, original draft preparation, supervision, editing. Research work conducted by scholar – Swarnalata Patnaik- red mud waste collection, field study, analysis and related laboratory experimental work. Smt. Patnaik contributed reagents, glassware, field related work, manuscript preparation, calculation and finalization of data. Prof. A.K. Panigrahi: Conceptualization, planning, supervision, field visit, script preparation, reviewing and editing.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.