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# Impact Of Red Mud Waste Extract On The Seed Biology Of A Green Gram Crop Seed Under Controlled Conditions.

**Swarnalata Patnaik and A.K.Panigrahi**

Mahamayee Mahila Mahavidyalaya, Berhampur-760001, Odisha. India.

*Environmental Toxicology Laboratory, Department of Botany, Berhampur University, BERHAMPUR-760 007, Odisha, India.

**Abstract**

The extract of the red mud waste prepared in the laboratory is almost equivalent to the lechate leaking from red mud pond of the contaminated site. The red mud waste extract is deadly toxic to the germinating crop seeds in field conditions. Experiments were conducted at sub-lethal concentrations to study the impact of lechate waste on green gram seed biological parameters. Toxicity study indicated that lechate extract is deadly toxic. In petriplate culture experiments the lechate waste is deadly toxic but toxicity depleted when tested in pot culture due to interaction of lechate waste with soil particles. Seed germination and seedling establishment significantly depleted with the increase in lechate concentration when compared to control seedlings. The total chlorophyll, pheophytin and carotene content of green gram plant leaves decreased significantly with the increase in lechate concentration compared to their respective control values after 144hr of germination. The photosynthetic rate, respiration rate and GPP values significantly decreased in the exposed green gram seedlings with the application of lechate compared to control green gram seedlings in petri plate culture. The residual alumina accumulation increased with the increase in toxicant (lechate) concentration in the leaves and roots. Lechate exposed roots accumulated more amount of alumina than the leaves of the exposed seedlings/ plants. The complex bio molecules like DNA, RNA, Protein, FAA content decreased in lechate exposure seedlings compared to control seedlings The photosynthetic rate, respiration rate and gross primary production significantly declined in exposed seedlings compared to control seedlings. Little insignificant increment in the photosynthetic rate was noted at sub-lethal concentration of the lechate. The enzyme activity tested decreased in the exposed green gram seedlings with the application of lechate compared to control green gram seedlings in Petri plate culture. The study on seed biology indicated that the red mud waste dumped by the industry is deadly toxic and if released accidentally even into the environment, will cause environmental havoc. Present study aims at understanding the impact of RM (red mud**)** RM Waste lechate on the seed germination, seedling establishment, eco-physiological studies, biomolecular studies and enzymological studies of a crop seed germinating under lechate stress.

**Key words:** Alumina, Lechate, Green Gram, Germination, Pigments, Photosynthetic efficiency, Biomolecules, Enzyme.

## Introduction

Industry is responsible for creating a fantastic array of new chemicals every year all of which eventually find their way into the environment. At present, the industry is the focus of attention, the world-over, as the strong polluter of the aquatic environments. Industries release large quantities of chemicals in the form of gas (exhaust), liquid (effluent) and solid wastes, into the environment. Many of these chemicals are toxic and create pollution problem. The problem of toxic hazard has already reached alarming proportions in this country and is bound to grow with increasing industrialization. Alumina industry is the main centre of air, water and soil pollution ((Patnaik *et al*, 2017, 2018). 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 at different stages of manufacture. The presence of rare earth and radioactive elements makes the task very difficult for safe disposal of red mud in many alumina-aluminum producing countries. The red mud waste comes out in the form of fine slurry with high alkalinity. The lechate chemicals are also highly alkaline and their entry into any crop field can imbalance the pH of the soil of the crop field. The red mud waste discharged from NALCO, Damonjodi contains a significant amount of alumina along with other chemicals. This chemical gets enriched to higher values in the red mud pond with time. Significant amount of alumina gets leached as lechate from the red mud pond and this lechate passes as a small stream from the red mud waste dumping site (RMP) and enters into nearby crop fields, where either rice / ragi is cultivated in rainy season followed by green gram or black gram as the second crop (Patnaik *et al*, 2022, 2023 and Leelaveni *et al*, 2023, 2024). Reports pertaining to impact of red mud waste on toxicity and seed biological parameter studies of crop plants are lacking (Patnaik *et al*, 2024). Present study aims at understanding the impact of RM (red mud) waste lechate on the eco-physiological, biochemical and enzymological activity of a crop seed germinating and establishing under stress.

**Location of the industry:**

NALCO Damanjodi, Koraput district, Odisha is located at latitude $18^0$-6'-$18^0$-58' towards North and longitude $82^0$.57'-$83^0$.04' East. ((Patnaik *et al*, 2022, 2023)

**(Photo- Industry & RM Pond down stream area showing wastes and leaching of waste)**

**Materials & Methods**

Red mud waste extract: The extract was prepared as explained by Patnaik *et al*., (2023) in the laboratory and used for the experiments. 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 of the waste, which is used for e experiments. This is the red mud extract RME or RMWE and known as the test toxicant.

*Vighna radiata*,(L.) Wilczek. Pure line uncontaminated seeds of green gram were obtained from Pulses and Millet Research Station, OUAT, Ratanpur, Ganjam.

The amount of total chlorophyll and total pheophytin 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 in a Photo-Warburg's apparatus (New Paul, India) following the procedure of Hannan and Patouillet (1972) and Oser (1965). Protein was determined by the procedure developed by Lowry *et al*. (1951), using a spectrophotometer. Inorganic phosphate produced was measured by the method of Fiske and Subbarow (1925) as modified by Martinek (1970).

**Results**

The red mud waste collected from Damonjodi was analyzed. The physical and chemical properties of the red mud waste and alumina hydrate was studied and published earlier by us (Pattanaik *et al*., 2018, 2022).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: Physical properties: LOI ( 300-1000o C ) % = 0.6-0.8 (Typical); 0.5-1.0 (Guaranteed range); BET surface area(M2/gm) =60-70 (Typical); 60-80 (Guaranteed range); Alpha Aluminium content %=10Max; Granulometry- +125 Micron(%)=15MAX ; - 45 Micron (%)= 10-

12MAX (GR); Chemical properties: $Al_2O_3$(%)=98.7 (T), 98.5 (Min); $Na_2O$(%)=0.38(T), 0.5(Min); $Fe_2O_3$(%)= 0.01(T), 0.015 (Max); $SiO_2$(%)= 0.012(T), 0.02 (Max); CaO(%)=0.042 (T), 0.050 (Max). Alumina hydrate: Physical properties: LOI (110-1000oC)%= 34-36 (T), 36 (Max); Moisture= 3-6 (T), Maximum=8; Granulometry:- 45 Micron(%)=3-6(T), Maximum=7

Chemical properties: $Al_2O_3$(%)=65+0.5(T),64(Min/Max);$Na_2O$(%)Total=0.23-0.30(T), 0.33(Max); $Na_2O$(%) Soluble=0.015-0.025(T), Max-0.30; $SiO_2$ (%)= 0.007-0.010(T), 0.016 Max $Fe_2O_3$(%)= 0.006-0.008(T), 0.016 (Max) ; $SiO_2$(%)= 0.012(T), 0.02 (Max); CaO(%)=0.042 (T), 0.050 (Max) ((Patnaik *et al*, 2017, 2018, 2022). The red mud waste was taken and an extract was prepared as described earlier. Red Mud Waste Extract prepared in the laboratory (1kg dry red mud powder with 2 liter distilled water-stirred for 15days at intervals and finally the RMWE is decanted and sieved and kept in refrigerator). The selected sub-lethal concentration for the study from toxicity testing was 12.5ml / liter (12.5ppm). Toxicity values obtained for green gram seed germination and seedling establishment in petri plate culture experiments: $LC_0$=0.075ml/liter (v/v); $LC_{05}$=0.2ml/liter (v/v); $LC_{10}$=1.5ml/ liter (v/v); $LC_{50}$=3.0ml/liter (v/v); $LC_{90}$=5.0ml/liter (v/v) and $LC_{100}$=5.6 ml/liter (v/v). From the toxicity test, it was found that all the seeds germinated (100%) up to 0.2% of the lechate.

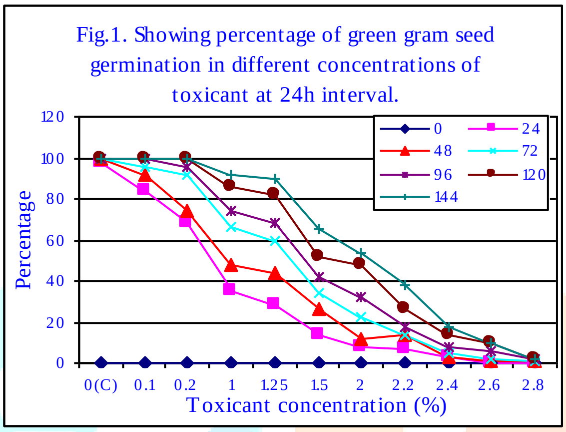

Fig.1. Showing percentage of green gram seed germination in different concentrations of toxicant at 24h interval.

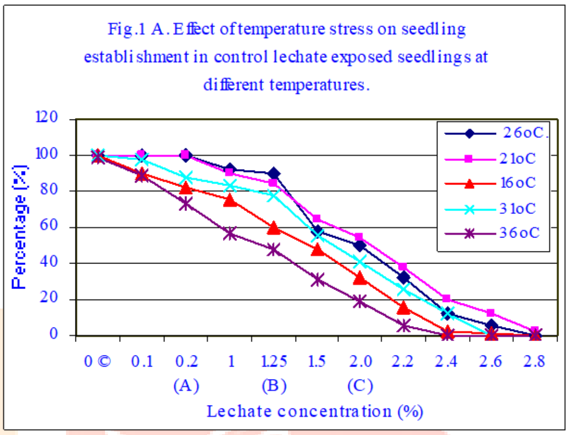

Fig.1 A. Effect of temperature stress on seedling establishment in control lechate exposed seedlings at different temperatures.

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. The changes in pigments content of control and leached chemicals of the red mud waste extract / lechate exposed seedlings of a green gram plant at different concentrations of the toxicant, after 144 hour of exposure.

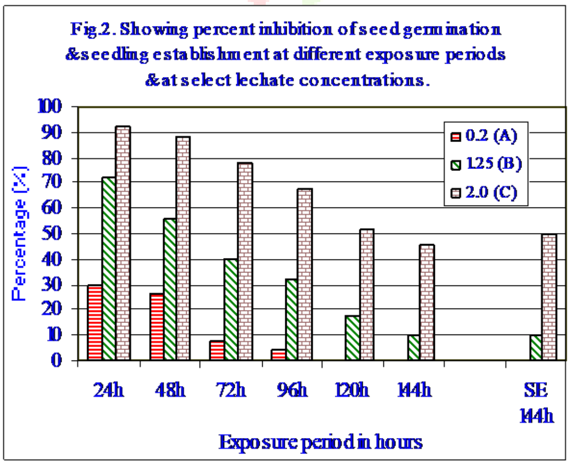

Fig.2. Showing percent inhibition of seed germination &seedling establishment at different exposure periods &at select lechate concentrations.

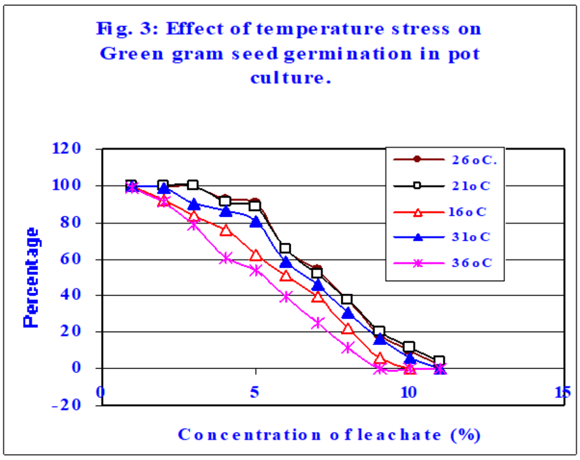

Fig. 3: Effect of temperature stress on Green gram seed germination in pot culture.

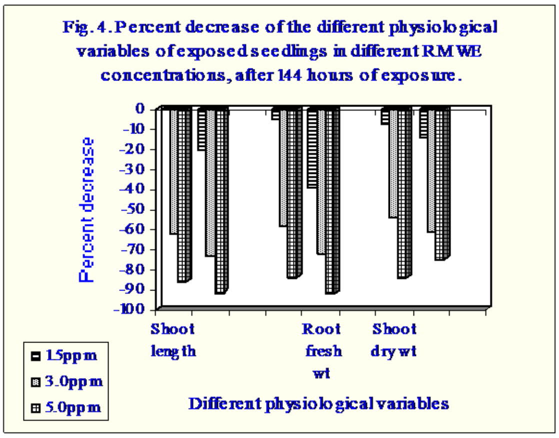

Fig. 4. Percent decrease of the different physiological variables of exposed seedlings in different RMWE concentrations, after 144 hours of exposure.

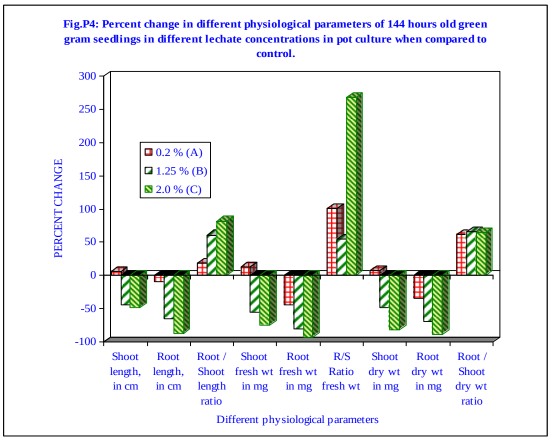

Fig.P4: Percent change in different physiological parameters of 144 hours old green gram seedlings in different lechate concentrations in pot culture when compared to control.

The accumulation of alumina was highest in exposed roots compared to shoots. Exposed roots accumulated 2.32±0.51µg/g dry weight after 144hrs of exposure at 1.5mg/liter lechate concentration in petri plate culture. Exposed roots accumulated 4.32 ± 0.36µg/g dry weight after 144hrs of exposure at 3.0mg/liter lechate concentration in petri plate culture. Exposed roots accumulated 5.14±0.71µg/g dry weight after 144hrs of exposure at 5.0mg / liter lechate concentration in petri plate culture (Fig.6). The shoot and root of the control green gram seedlings did not show any accumulation of alumina indicating no contamination and served as standard control for comparison. No alumina was recorded in the control set green gram plant leaves in pot culture. The leaves of red mud waste lechate exposed green gram plants accumulated 1.24±0.34µg/g dry weight; 2.42±0.38µg/g dry weight; 4.38±0.54µg/g dry weight and 5.26±0.66µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 1.5mg/liter lechate concentration (Fig.7). The leaves of lechate exposed green gram plants accumulated 2.38±0.52µg/g dry weight; 3.65±0.24µg/g dry weight; 5.91±0.38µg /g dry weight and 8.45±1.12µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 3.0mg/liter lechate concentration. The leaves of lechate exposed green gram plants accumulated 2.71±0.46µg/g dry weight; 5.28±0.65µg/g dry weight; 7.34±0.46µg/g dry weight and 9.98±1.44µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 5.0mg/liter lechate concentration (Fig 7). The residual accumulation of alumina increased in lechate exposed green gram plant leaves with the increase in exposure period in pot culture. The root of lechate exposed green gram plants accumulated 2.14±0.61µg/g dry weight; 2.95±0.46µg/g dry weight; 4.65±0.66µg /g dry weight and 5.69±0.71µg /g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 1.5mg/liter lechate concentration. The residual accumulation of alumina increased in lechate exposed green gram plant roots with the increase in exposure period in pot culture. The roots of lechate exposed green gram plants accumulated 2.88±0.45µg /g dry weight; 4.12±0.52µg/g dry weight; 6.88±0.32µg/g dry weight and 9.11±0.68µg /g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 3.0mg/liter lechate concentration. The roots of lechate exposed green gram plants accumulated 3.26±0.52µg/g dry weight; 5.98±0.34µg/g dry weight; 7.28±0.69µg/g dry weight and 11.55 ± 0.64µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 5.0mg/liter lechate concentration (Fig. 7).

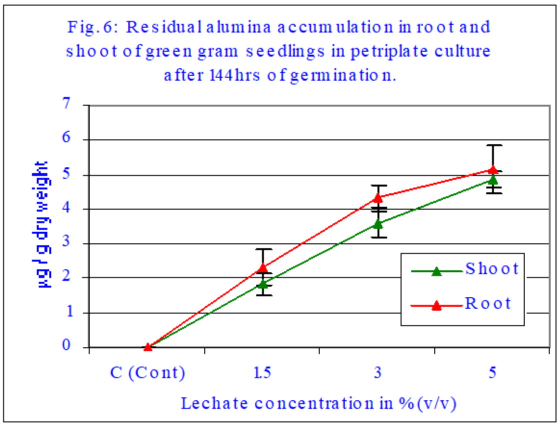

Fig.6: Residual alumina accumulation in root and shoot of green gram seedlings in petriplate culture after 144hrs of germination.

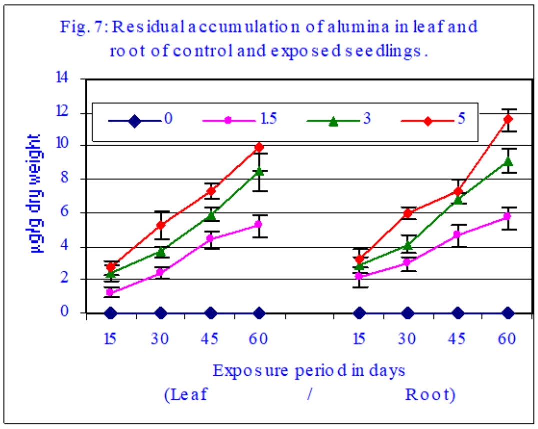

Fig. 7: Residual accumulation of alumina in leaf and root of control and exposed seedlings.

It was observed that the pigments, physiological parameters and enzyme activity decreased with the increase in alumina concentration in germinating seedlings. The chlorophyll-a content decreased steadily and linear. A maximum of 89.3% decrease was recorded at higher concentration. The chlorophyll-b content decreased at conc. A & B and significant decrease in conc.-C, where a maximum of 96.3% decrease was recorded at conc. C of the toxicant. The total chlorophyll content decreased by 92.4%, compared to control values (Fig.8, 9&10). The total pheophytin content decreased by 91.7% at conc.-C when compared to the control value. Interesting changes were observed in rate of respiration, rate of photosynthesis and Gross Primary Productivity in control and lechate exposed 144-hour-old seedlings in plate culture, grown in a seed germinator under controlled conditions. Change in photosynthetic rate, respiration rate and GPP values at different concentrations of the lechate and at different days of exposure in pot culture indicated interesting features. The photosynthetic rate decreased by 50.6% (fig.11).

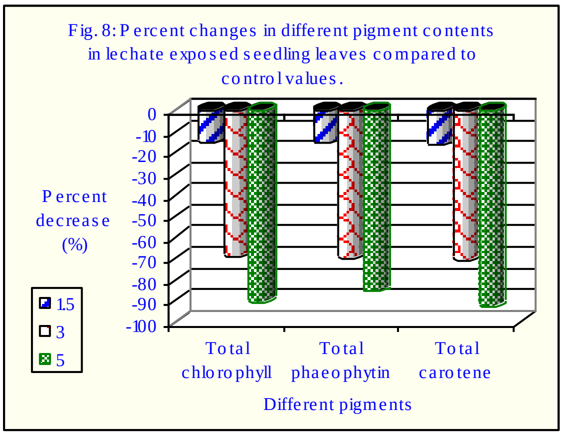

Fig. 8: Percent changes in different pigment contents in lechate exposed seedling leaves compared to control values.

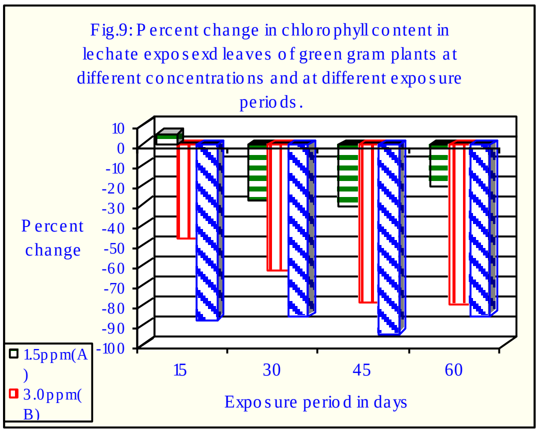

Fig.9: Percent change in chlorophyll content in lechate exposexd leaves of green gram plants at different concentrations and at different exposure periods.

On 30th day of exposure, the rate of photosynthesis value depleted by 56.9%, On 45th day of exposure, the rate of photosynthesis value depleted by 64.6% decrease was recorded and on 60th day of exposure, the rate of photosynthesis decreased by 77.2% at conc.-C. The rate of respiration decreased from 236.5±18.4 to 208.4±19.6µl of $CO_2$ evolved $hr^{-1}$ $g^{-1}$ dry weight of the seedling at conc. A, from 236.5±18.4 to 102.6±28.8µl of $CO_2$ evolved $hr^{-1}$ $g^{-1}$ at conc.-B and from 236.5±18.4 to 64.5±12.6µl of $CO_2$ evolved $hr^{-1}$ $g^{-1}$ at conc.-C in 144 hour old rice seedlings (Fig.11).

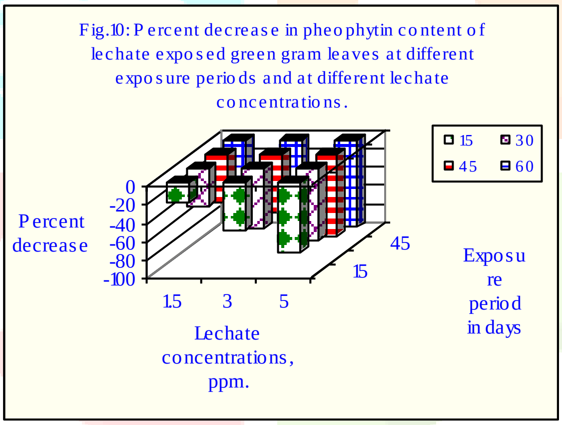

Fig.10: Percent decrease in pheophytin content of lechate exposed green gram leaves at different exposure periods and at different lechate concentrations.

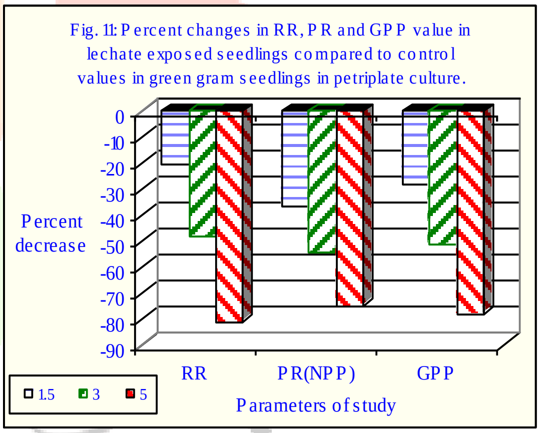

Fig. 11: Percent changes in RR, PR and GPP value in lechate exposed seedlings compared to control values in green gram seedlings in petriplate culture.

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 40%, 45.7% and 68.6% at conc. A, conc. B and conc. C after 45days of exposure in the lechate exposed leaves of green gram plants. The DNA content decreased by 48.7%, 51.4% and 86.5% at conc. A, conc. B and conc. C after 60days of exposure in the lechate exposed leaves of green gram plants (Fig.12). 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). he RNA content increased by 6.9% at sub-lethal conc. A on 15th day of exposure in lechate exposed green gram plants in pot culture. With the increase in lechate concentration, the RNA content decreased by 21.6% and 47.4% at conc. A, conc. B and conc. C after 15days of exposure in the lechate exposed leaves of green gram plants. The RNA content decreased by 8.2%, 22.9% and 55.7% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed leaves of green gram plants (Fig.12).

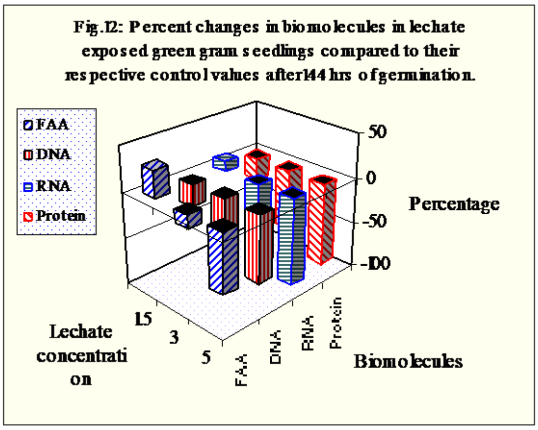

Fig.12: Percent changes in biomolecules in lechate exposed green gram seedlings compared to their respective control values after 144 hrs of germination.

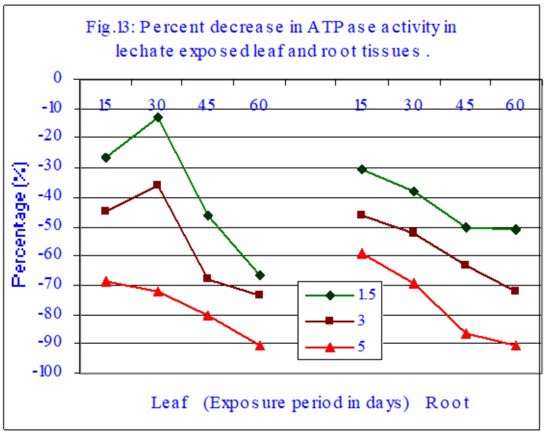

Fig.13: Percent decrease in ATPase activity in lechate exposed leaf and root tissues.

The protein content decreased by 16.5%, 37.4% and 64.1% at conc. A, conc. B and conc. C after 15days of exposure in the lechate exposed leaves of green gram plants. The protein content decreased by 28.8%, 46.2% and 71.2% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed leaves of green gram plants. The protein content decreased by 41.3%, 60.1% and 80.7% at conc. A, conc. B and conc. C after 45days of exposure in the lechate exposed leaves of green gram plants. The protein content decreased by 53.5%, 74.5% and 90.8% at conc. A, conc. B and conc. C after 60days of exposure in the lechate exposed leaves of green gram plants (Fig.12). e protein content decreased by 10.7%, 52.3% and 77.6% at conc. A, conc. B and conc. C after 15days of exposure in the lechate exposed roots of green gram plants. The protein content decreased by 39.9%, 59.2% and 86.1% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed roots of green gram plants. The protein content decreased by 58.4%, 69.8% and 95.5% at conc. A, conc. B and conc. C after 45days of exposure in the lechate exposed roots of green gram plants. The protein content decreased by 67.3%, 87.7% and 97.2% at conc. A, conc. B and conc. C after 60days of exposure in the lechate exposed roots of green gram plants (Fig.12). The FAA content increased by 30.4% at sub-lethal conc. A on 15$^{th}$ day of exposure in lechate exposed green gram plants in pot culture. With the increase in lechate concentration, the FAA content increased by 24.1% and decreased by 39.3% at conc. B and conc. C after 15days of exposure in the lechate exposed leaves of green gram plants. The FAA content increased by 22.6%, and then decreased by 4.8% and 50% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed leaves of green gram plants, respectively. The FAA content initially increased by 21.1% and then decreased by 40.6% and 60.9% at conc. A, conc. B and conc. C after 45days respectively, of exposure in the lechate exposed leaves of green gram plants. The FAA content increased by 22.5% at conc. A and then decreased by 64.1% and 80.3% at conc. B and conc. C after 60days of exposure respectively in the lechate exposed leaves of green gram plants (Fig.12). The FAA content increased by 14.5% at conc. A and then the FAA content decreased by 1.6% and 54% at conc. B and conc. C after 15days of exposure in the lechate exposed roots of green gram plants respectively. The FAA content decreased by 12.3%, 18.8% and 76.8% at conc. A, conc. B and conc. C after 30days of exposure in the lechate exposed roots of green gram plants. The FAA content decreased by 19.1%, 24.8% and 90.1% at conc. A, conc. B and conc. C after 45days of exposure in the lechate exposed roots of green gram plants, respectively. The FAA content decreased by 27.8%, 39.1% and 92.1% respectively, at conc. A, conc. B and conc. C after 60days of exposure in the lechate exposed roots of green gram plants (Fig. 12). ATPase an important enzyme playing a vital role in energy metabolic processes was considered for study to understand the role played by the toxic wastes on physiological processes of energy molecule build up and energy molecule break up to release energy for conducting different processes. The changes in ATPase activity of 144-hour-old green gram seedlings in different concentrations of the toxicant in petriplate culture, grown in a seed germinator under controlled conditions showed features related to toxicity. The ATPase activity of the 144-hour-old green gram seedlings, decreased with the increase in toxicant concentration, when compared to the control value. The ATPase activity significantly decreased in lechate exposed green gram plant roots at all exposure periods and at all select lechate concentrations in pot culture. The ATPase activity decreased by 42.1% in conc. A, 71.1% in conc. B and 86.8% decrease in conc. C, respectively in red mud waste lechate exposed green gram seedlings, when compared to their respective control values. After 60days of exposure the ATPase activity decreased by 51.2%, 72.1% and 90.7% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram leaves compared to control values (Fig.13).

**Discussion:**

Due to extremely high demand for aluminium all over the world, red mud generation by Alumina industries has been reached to more than 150 million tones per year globally. Xue *et al* (2016b) reported that "disposal of such huge quantity of wastes includes marine/slurry disposal, dry stacking and dry cake stacking. At present, dry cake stacking is highly adopted as it attempts to produce 65-70% solid cake before disposal which minimizes land acquisition and reduces the risk of environmental contamination". "Improper disposal of poorly treated residue may lead to several environmental problems with its consequent impacts on living beings. The red mud waste coming out from the industry at Damonjodi is discharged as slurry into a red mud pond created nearby surrounded by hills from all sides except one opening where the industry owners closed the opening by an earthen dam. The slurry is allowed to dry in the pond. The lechate of the red mud pond have contaminated the surrounding area, where all forms of plant and animal life suffer significantly. There is no single well defined technique adopted by the industries. Every industry uses Bayer's protocol for extraction of Aluminium where bauxite ore is treated with hot NaOH (caustic soda) under very high temperature and pressure. Liu *et al* (2009) reported that "approximately 85-90% of bauxite is converted to alumina followed by Hall-Heroult process for aluminium production". Xue *et al* (2016b) and Wang *et al.*, (2008) indicated that many advanced red mud management protocols like catalytic application processes, metallurgical processes, manufacture of paints and pigments, adsorbents, ceramics, and construction materials. The above options are cost-prohibitive and they are also not eco-friendly and require much polishing and refinement of the wastes before disposal to natural environments. Instead of any type of treatment, the best method of treatment was suggested by Xue et al (2016b) pertaining to development of a long lasting and cost effective suitable vegetation cover over red mud dam. Simply this method is not acceptable as the red mud waste is deadly toxic and showed adverse effects on flora and fauna of the area. For this type of method a counterbalanced technique to counter the toxic nature of red mud waste should be adopted first by way of using suitable amendments or by natural ways. The plant over or the vegetation of an area indicates the soil status and condition and any change in the physico-chemical status will alter the vegetation composition and ultimately plant cover. The red mud waste has many nutrients used by plants but an addition to nutrients, the red mud waste contains many toxic chemicals comparatively in huge quantities which can significantly affect plant growth, survival and development. At present China ranks first in alumina production and consumption in the world. Accordingly China also the highest producer of red mud waste out of which only 4% is being utilized. Most of the industries adopt Bayer's process for production of alumina. In this process red mud waste is generated. The red mud waste is discharged and stored in a red mud pond well guarded by natural walls like hills and mountains and man created / constructed dams. The industry people remove the dried red mud waste and periodically and these dried red mud waste is used for construction of new roads. Most of the countries adopt the method of stacking of red mud waste in storage tanks (red mud pond) or centralized stocking in the form red cakes as bulk deposits. Whatever may be the type of technology except alumina the rest of the chemicals are discharged as a waste, except in few industries where few more chemicals were isolated, purified and separated. It depended upon the type bauxite mine deposits. These red mud wastes destroy the soil structure and quality, the leached chemicals of the red mud pond destroys the physico-chemical quality of water bodies. When all the data were considered along with the statistical data it can be inferred that the toxicant was more effective in Petri plate culture, when compared to pot culture experiment. Higher percent of inhibition was recorded in Petri plate culture experiments. Petri plate experiments were conducted in side a seed germinator, where all environmental factors were controlled. But 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 in field conditions could have provided much better information. But due to lack of facilities and equipments, residual analysis part was not carried out by the scholars. The present study on residual accumulation of alumina was carried out to understand the entry of alumina in to green gram seedlings. The red mud wasie extract contained alumina but in addition, so many other metals were present. It was not practically possible to study all the chemicals that were absorbed by the green gram seedlings due to lack of infrastructure and instrumental facilities. In presence of residual accumulation of toxicant data, the obtained data can be analyzed, correlated and interpreted properly. At present all the obtained data were correlated to alumina residue only. However, in absence of other metal residual accumulation data, concrete conclusions may not be drawn but to a greater degree of accuracy, the data was presented and interpreted. All the obtained data were statistically significant. Further, higher-level work is necessary to assess the toxic nature and site of the action studies. 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. The study on seed biology indicated that the red mud waste dumped by the industry is deadly toxic and if released accidentally even into the environment, will cause environmental havoc. Present study aims at understanding the impact of RMP (red mud pond) lechate on the biological activity of a crop seed germinating under stress (Patnaik *et al*, 2017, 2018, 2022, 2023a, 2024 and Leelaveni *et al*, 2023). The data indicated drastic decline in seed germination, seedling establishment, root and shoot growth and pigments in RM Waste lechate compared to respective control values. The spectral analysis study indicated depletion in peak values of the pigments in exposed seedling shoot extracts, when compared to control seedling shoot pigment extracts at lower concentration of red mud waste. The acetone pigment extract of the control seedlings showed a normal feature in the scanogram. The depletion of pigment content in the red mud waste exposed seedling was well evident from spectral analysis of pigments. At higher concentration of red mud waste, the pigment peaks altogether disappeared. The disappearance of pigment peak indicated the destruction of most important pigments at higher concentrations of red mud waste. No shift of peak for any pigment was noticed. This indicated no change in the characteristic feature of the pigments induced by the toxicant, red mud waste of the Alumina industry situated at Damonjodi. 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 chl-a, chl-b, total chlorophyll, pheophytin and carotene content. The accumulation of alumina was highest in exposed roots compared to shoots. The shoot and root of the control green gram seedlings did not show any accumulation of alumina indicating no contamination and served as standard control for comparison. The root of the lechate exposed seedlings showed the maximum alumina deposition compared to stem and leaf. 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. All the data presented showed similar trend in all the parameters of physiological variables studies. It was interesting to note that at 0.2% lechate concentration, the physiological variable showed a higher value when compared to the control value. Whereas, with the increase in lechate concentration (1.25%- Conc. B and Conc. C -2.0%), the physical parameters decreased significantly when compared to the control value. Increase in 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 exposed roots were more affected than the shoot. 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 observed that in the early phases of germination, when the roots came out, these roots were moving upwards away from the toxicant. The red mud waste is a complex mixture of more than 87 metals and 137 compounds as reported earlier. Mohammed *et al*., (2019) reported that heavy metal pollution of Egyptian water was primarily because of agricultural and industrial wastes and fish was the main sufferer in water bodies. Cuciureanu *et al*., (2020) reported that red mud was the main waste produced during alkaline leaching of bauxite ore and a large quantity of waste was generated in the process and were discharged on large areas year by year needs attention. As these wastes can cause serious environmental and health issues, the author tried to reuse or recycle the red mud wastes for a green future. Patel and Pal (2015) reported that the studied red mud waste was strongly alkaline ranging between 10 to 13 and warned that these wastes were corrosive in nature can cause alarming environmental problems. Murali *et al*., (2018) investigated the toxicological impacts of $Al_2O_3$ nanoparticles on histoarchitecture of fresh water fish at sub-lethal concentrations and found loss of cellular architecture because of the toxicant application. Cui *et al*., (2019) reported the leaching behavior of metal elements of red mud was controlled by solubility and not by the concentrations of these metal elements in the red mud waste. This idea can be used while studying the impact of red mud waste on any aquatic animal or plant, as these elements can only be absorbed from the waste, if they are available in the soluble form. Sun *et al*., (2019) studied the geochemical characteristics and presence of toxic elements in alumina refining waste and lechate coming from management facilities and reported the presence of minor elements and trace elements in the waste and these wastes can be toxic to aquatic life due to hyper alkaline nature of red mud lechate. Zhou *et al*., (2018) reported selective leaching of scandium from red mud and opined that selective leaching is important for reclamation and solid waste treatment while working on red mud waste reclamation. Sarath Chandra and Krishnaiah (2018) conducted a detailed geotechnical analysis of red mud and reported that the waste is iron-oxide rich and commented that the storage and disposal of red mud is the biggest problem and this red mud waste seriously contaminates the aquatic ecosystems. The surrounding areas near industrial establishments may show higher levels of metals via aerial deposition in particulate form. From the data it is well evident that though no residual accumulation of alumina or non detectable amount of alumina was observed in the seeds of the green gram crop plants but the vegetative biomass contained residual alumina and hence this crop can be recommended for cultivation but the vegetative biomass should not be allowed to be grazed or eaten by grazers or herbivores as alumina might accumulate in the body tissues of grazers and can cause serious illness among them.

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