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Physiological And Biomolecular Studies Of Green Gram Seedlings Impacted By Red Mud Waste Discharged From Alumina Industry In 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 red mud pond flows as a small canal and contaminates the river. The lechate contaminates the ground water of the contaminated site and neighboring crop fields. 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 in RMWE exposed seedlings.
- The leached chemicals / RMWE significantly affected the pigment contents of the exposed seedlings and altered the pigment ratio values of the seedlings
- RMWE influenced the physiological activities and biomolecular content of the exposed crop seedlings thereby reducing the productivity of the crop plant.
- Significant increase in residual alumina content was noted in RMWE exposed seedlings. The residual alumina content affected the studied parameters.

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. From the present study and the results observed from the study of other researchers of our laboratory indicated that the RMWE is deadly toxic and the rice crop should be replaced with green gram crop. It was observed that lechate leaking from the red mud pond flows as a small canal and contaminates the river. The lechate contaminates the ground water of the contaminated site and neighboring crop fields. 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 in RMWE exposed seedlings. The leached chemicals / RMWE significantly affected the pigment contents of the exposed seedlings and altered the pigment ratio values of the seedlings. RMWE influenced the physiological activities and biomolecular content of the exposed crop seedlings thereby reducing the productivity of the crop plant. Significant increase in residual alumina content was noted in RMWE exposed seedlings. The residual alumina content in exposed root and shoot affected the studied parameters.

Keywords: Red mud waste, RMWE, crop plant, seed biology, pigment, GPP, biomolecules.

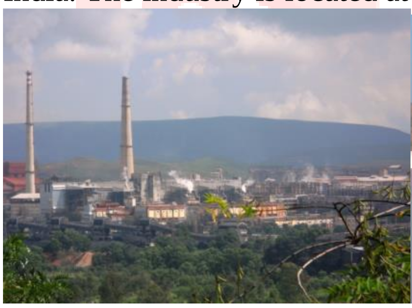
Introduction

The NALCO 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). Alumina industry is the main centre of air, water and soil pollution of the surrounding area. 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 waste chemicals as gases or liquids or solids at different stages of manufacture. The gases are generally released in to air through chimneys which causes air pollution. The liquid waste is very thick (red mud=RM) called as slurry is discharged into a stocking area (RM Pond) surrounded by natural hills from all sides and a small link is left out which is closed by earthen dam in the form of a dam for air drying of the red mud waste. Earlier probably no body thought of leakage and leaching of waste from the red mud pond located at Damonjodi, Koraput district. Neither the pond is plastic lined nor cemented, hence 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 liquid waste is leaking 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 and contaminated. 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 crop fields and water bodies. 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 reservoirs. The 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; Lechate leaking from the RMP of the industry)

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

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- +125Micron(%) = 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)

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 and RNA by Orcinol reagent method of Volkin and Cohn (1954). The amount protein was estimated by the procedure of Lowry *et al*. (1951) & the protocol followed by Radha (2004). FAA content was estimated by Ninhydrin method following the procedure of Lee & Takahasi (1966). All the obtained values were statistically analyzed.

Results

Toxicity study is one of the very important aspects of pollution studies. It is necessary to first determine the safe level concentration of the pollutant / toxicant for conducting any study. In the present toxicity test, it was found that all the seeds germinated (100%) up to 0.2% of the lechate (RMWE). 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 ($r=0.955$; $p\leq0.01$). 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 (Fig.1). From the data, it can be inferred that higher doses / concentrations of red mud lechate 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. 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 (Fig.1).

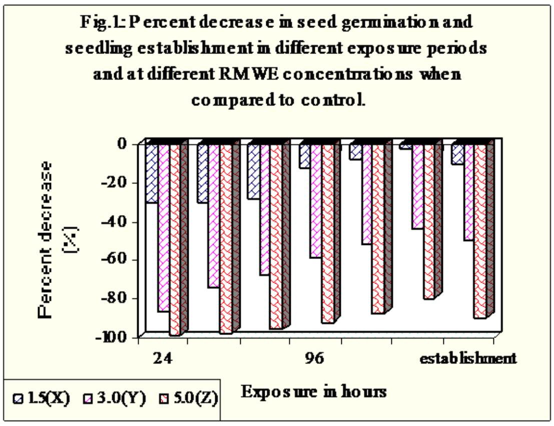


Fig.1: Percent decrease in seed germination and seedling establishment in different exposure periods and at different RMWE concentrations when compared to control.

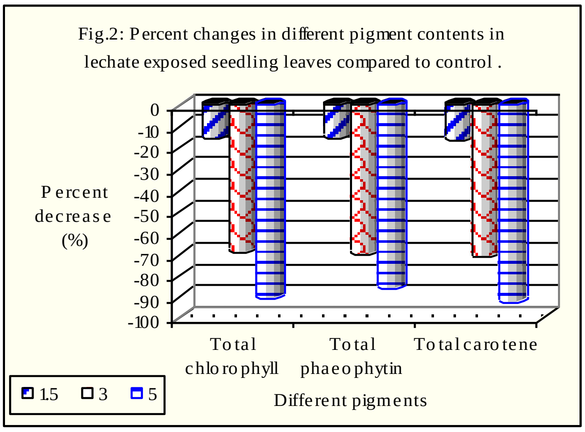


Fig.2: Percent changes in different pigment contents in lechate exposed seedling leaves compared to control .

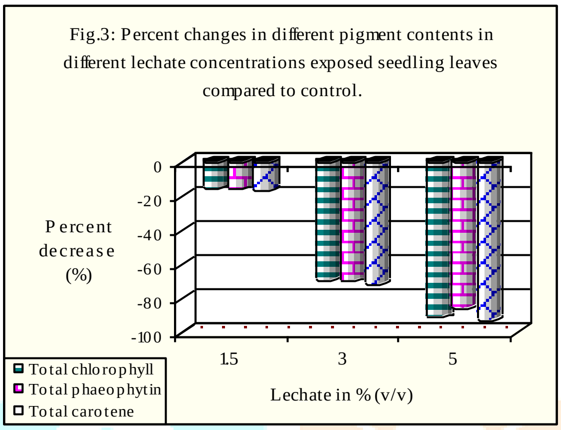


Fig.3: Percent changes in different pigment contents in different lechate concentrations exposed seedling leaves compared to control.

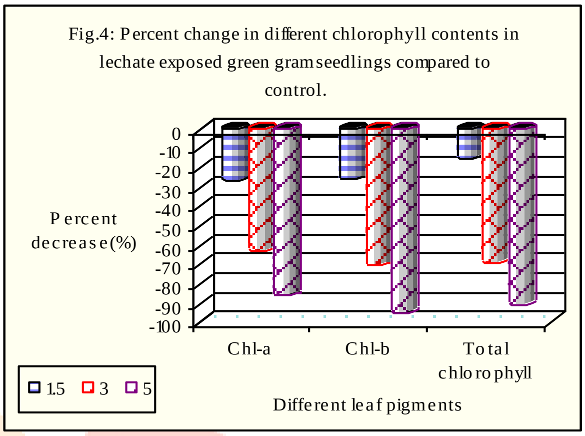


Fig.4: Percent change in different chlorophyll contents in lechate exposed green gram seedlings compared to control.

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 in petriplate culture. The chlorophyll-a content increased from 0.566±0.08mg/g fresh weight to 0.58±0.11mg/g fresh weight at conc.-A, decreased from 0.56±0.08mg/g fresh weight to 0.22±0.04 mg/g fresh weight at conc.-B, and from 0.56±0.08mg/g fresh weight to 0.06±0.02mg/g fresh weight at conc. C. The decrease was steady and linear. A maximum of 89.28% decrease was recorded at con. C. The chlorophyll-b increased from 0.54±0.11mg/g fresh weight to 0.59±0.06mg/g fresh weight at conc. A; decreased from 0.54±0.11mg/g fresh weight to 0.19±0.22mg/g fresh weight at conc. B and from 0.54±0.11mg/g fresh weight to 0.22±0.01mg/g fresh weight at conc.-C, where a maximum of 96.3% decrease was recorded at conc.-C of the toxicant. The total chlorophyll content increased from 1.19±0.22mg/g fresh weight to 1.24± 0.31mg/g fresh weight at conc. A; decrease from 1.14±0.3mg/g fresh weight to 0.48 ± 0.2mg/g fresh weight at conc. B and from 1.19±0.22mg/g fresh weight to 0.09±0.2mg/g fresh weight at conc.-C after 144 hours of exposure (Fig.2). A maximum of 92.44% decrease in total chlorophyll content was recorded at concentration C, when compared to the control value. The total phaeophytin content decreased from 0.96±0.06mg/g fresh weight to 0.91 ± 0.04mg/g fresh weight at conc.-A; from 0.96±0.06 mg/g fresh weight to 0.21±0.05 mg/g fresh weight at conc.-B and from 0.96±0.66 mg/g fresh weight to 0.08±0.01 mg/g fresh weight at conc.-C. A maximum of 91.67 % decrease was recorded at conc.-C when compared to the control value. The carotene decreased from 0.0146±0.0014 mg/g fresh weight to 0.0141±0.0008 mg/g fresh weight at conc.-B and from 0.0146±0.0014mg/g fresh weight to 0.0008±0.0001 mg/g fresh weight at conc.-C. A maximum of 94.52% decrease over the control value was recorded at conc.-C. The chlorophyll a/b ratio increased with the increase in toxicant concentration from 1.04 to 3.0. The total chlorophyll / phaeophytin ratio decreased from 1.24 to 1.12 at conc. C and the total chlorophyll / total carotene ratio increased from 81.51 to 112.5 at conc. C, where a maximum increase of 38.02 was recorded at conc. C. 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 (Fig.3) 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, phaeophytin and carotene content. The analysis of variance ratio test conducted basing on the data indicated the existence of significant difference between columns and non-significant difference between rows. 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. Chl-a decreased from 0.56mg.g^{-1} to 0.08mg.g^{-1} at 5ppm toxicant concentration, Chl-b decreased from 0.54mg.g^{-1} to 0.03mg.g^{-1} at 5ppm toxicant concentration, Chl-a decreased from 0.56mg.g^{-1} to 0.08mg.g^{-1} at 5ppm toxicant concentration, total chlorophyll decreased from 1.19mg g^{-1} to 0.11mg g^{-1} at 5ppm toxicant concentration (Fig.4). Total pheophytin decreased from 0.96mg g^{-1} to 0.014mg g^{-1} 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.5). The Ratio value showed an increase in chlorophyll a/b ratio value (Fig.5). The Chlorophyll/ phaeophytin ratio value decreased with the increase in toxicant concentration (Fig.5). 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. Fig. 6 & 7 showed the changes in biomolecules in RMWE / lechate exposed seedlings of green gram in petriplate culture. Significant decrease in DNA, RNA, protein and FAA content was noted in red mud waste extract exposed green gram seedlings compared to the control seedlings. The decrease in DNA content was probable due to death of cells or tissues or interference of red mud waste chemicals in DNA synthesis. The decrease in RNA content was probably due to interference of red mud waste chemicals during RNA synthesis. The decrease in amino acid content in the red mud waste exposed seedlings when compared to control seedlings was probably due to inhibition of amino acid synthesis. The decrease in protein content was either due to proteolysis or due to non synthesis of proteins. The impact was severe at higher concentrations of red mud waste extract / lechate.

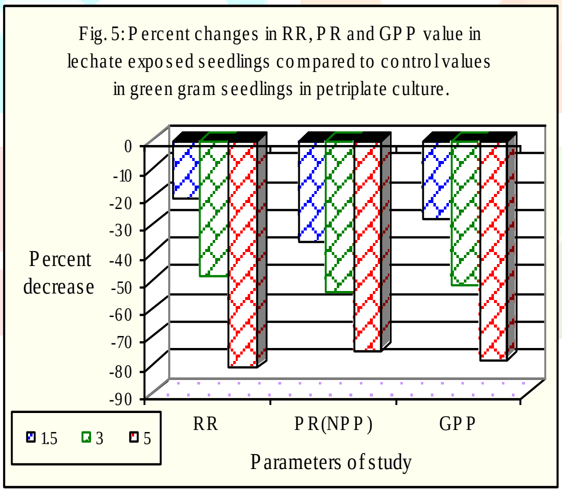


Fig. 5: Percent changes in RR, PR and GPP value in lechate exposed seedlings compared to control values in green gram seedlings in petriplate culture.

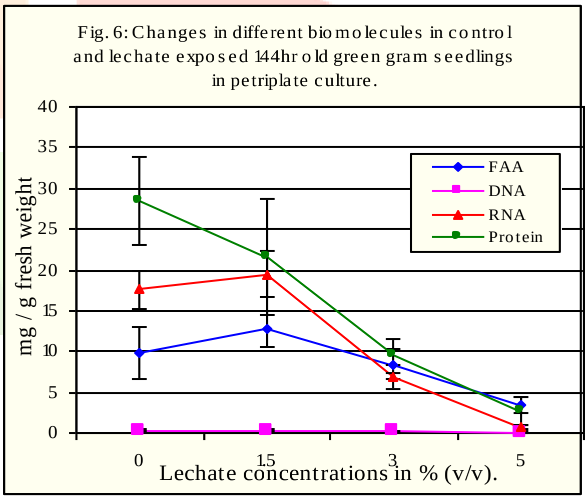


Fig. 6: Changes in different biomolecules in control and lechate exposed 144hr old green gram seedlings in petriplate culture.

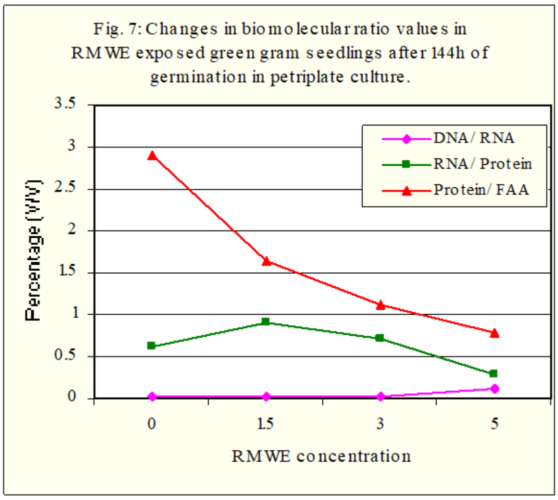


Fig. 7: Changes in biomolecular ratio values in RMWE exposed green gram seedlings after 144h of germination in petriplate culture.

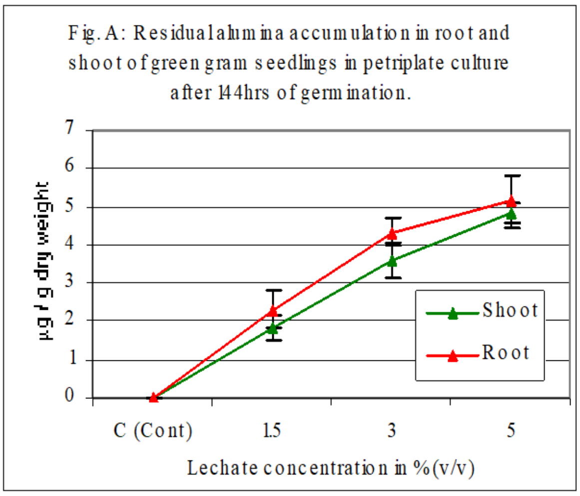


Fig. A: Residual alumina accumulation in root and shoot of green gram seedlings in petriplate culture after 144hrs of germination.

Fig. A showed the changes in residual accumulation of alumina in shoot and root of 144hr old green gram seedlings in petriplate culture exposed to different concentrations of the lechate of red mud pond waste collected from NALCO, Damonjodi. 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 petriplate culture. Exposed roots accumulated 4.32±0.36µg / g dry weight after 144hrs of exposure at 3.0mg/liter lechate concentration in petriplate culture. Exposed roots accumulated 5.14±0.71µg / g dry weight after 144hrs of exposure at 5.0mg / liter lechate concentration in petriplate culture (Fig. A). 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. When all the data were considered along with the statistical data it can be inferred that the toxicant was more effective in petriplate culture.

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 (Evans, 2016). Xue *et al* (2021) 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 (Mayes *et al*., 2016). 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. Higher percent of inhibition was recorded in petriplate culture experiments. Petriplate 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 could have provided much better information. But due to lack of facilities and equipments, residual analysis part was not carried out. In presence of residual accumulation of toxic ant data, the obtained data can be analyzed, correlated and interpreted properly. However, in absence of residual accumulation data, concrete conclusion 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. 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. Shetty *et al* (2020) reported decreased root elongation and shoot growth, depleted biomass production in red mud waste exposed plants. The same author also indicated that the depletion in the quality of the products of the plants and decrease in the production (final yield) as a consequence of red mud exposure. Singh *et al*, (2017) reported that Aluminium toxicity will interfere in nutrient absorption and most important nutrients like P, K, Ca, Mg, Mo, and B will not be absorbed, transported and utilized by the plants growing in red mud waste contaminated environments. 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). 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. 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. Change in photosynthetic rate, respiration rate and GPP values at different concentrations of the toxicant and at different days of exposure in pot culture indicated interesting features. The gross primary productivity of the exposed seedlings drastically reduced, when compared to the control value.

Biomolecular studies were conducted to understand the impact of RMW lechate in germinating green gram seedlings after 144hrs of exposure in different lechate concentrations in petriplate culture. The DNA content of 144hr old green gram seedling shoot decreased significantly. The DNA content in lechate exposed green gram seedlings decreased by 23.5%, 47.1% and 73.5% in conc. A, conc. B and conc. C respectively compared to the control values. The RNA content of 144hr old green gram seedling shoot increased at 1.5ml/liter lechate concentrations showing 10.8% increase over the control value. The DNA/RNA value decreased from 0.019 to 0.013 at 1.5ml/liter lechate concentration and the ratio value increased to 0.026 at conc. B and the ratio value further increased to 0.113. The decrease in ratio value at conc.-A and significant increase at higher lechate concentrations probably indicated the impact of RMW lechate at biomolecular level impacting DNA synthesis. It was not possible to draw any concrete conclusion from these values (Fig.31). The RNA/protein ration increased from 0.619 to 0.903 at conc. A and increased from 0.619 to 0.726 at conc. B and the ratio value significantly decreased to 0.296 from 0.619 at conc. C indicating that the toxicant RMW lechate probably affected the protein synthesis metabolism or induced proteolysis. The protein/FAA ratio notably decreased from 2.897 to 1.64 at conc. A, from 2.897 to 1.13 at conc. B and from 2.897 to 0.79 at conc. C indicating either proteolysis or impacting the protein metabolism leading to non production of proteins in 144hr old green gram seedlings exposed to RMW lechate in petriplate culture. In petriplate culture the impact of RMW lechate was direct. With the increase in lechate concentration, the RNA content of 144hr old green gram seedling shoot decreased in petriplate culture. The protein content of 144hr old green gram seedling shoot decreased at 3.0ml/liter lechate concentrations. The protein content of 144hr old green gram seedling shoot decreased at 1.5ml/liter lechate concentrations. The protein content in lechate exposed green gram seedlings decreased by 23.5%, 47.1% and 73.5% in conc. A, conc. B and conc. C respectively compared to the control values. The FAA content of 144hr old green gram seedling shoot increased at 1.5ml/liter lechate concentrations showing 30.6% increase over the control value in petriplate culture. With the increase in lechate concentration, the FAA content of 144hr old green gram seedling shoot decreased notably in petriplate culture. The RNA content in lechate exposed green gram seedlings decreased by 14.3% and 65.3% in conc. B and conc. C, respectively compared to the control values.

It was observed that heavy metals like mercury, cadmium, lead and metals like Aluminium at lower concentrations were absorbed faster when compared to higher concentrations of these metals. The absorption of these metals varies from species to species and from different genus to genus. Absorption and accumulation also varies from species to species. The absorption and accumulation is time dependent. The younger plants and particularly seedlings absorb metals faster than old plants. With the increase in age, the absorption and retention capacity decreases significantly. The absorbed chemicals can be excreted out but it depends on the translocation routes and site of accumulation. Wide species variations are recorded for the accumulative efficiency for different heavy metals. The difference in metal accumulation is not correlated with tolerance to the heavy metal. Heavy metals pose a number of hazards to human health. Therefore their concentration in the environment and their effects on human health must be regularly monitored. More researches are required to understand the mechanisms involved in heavy metal tolerance in plants. Metal induced defense response at molecular level need to be worked out for understanding the cascade of chemical mechanisms of heavy metal tolerance. In the present investigation, we have not seen such stimulatory affect. No doubt at very dilute concentrations stimulatory effect or increase in few parameters was noted in the exposed seedlings. The reason could be that the red mud waste extract or lechate contained many chemicals which are required for the plant growth and development and these chemicals were the part of micro and macronutrients generally supplemented for better growth of the plants during germination, seedling establishment and plant growth and flowering. Basing on these observations we can not recommend these waste chemicals for use in crop biological studies. It is well understood that this toxicant is deadly toxic and recommended dilutions as per toxicity study is only practicable in laboratory studies but not in field conditions. The amount of red mud waste load b y the industry is so high that neither we can suggest dilution of the wastes or use of these diluted wastes for use in agriculture, where huge amount fresh water will be used up nor we can not afford such a huge amount of fresh water use from natural rivers. Even if we dilute for protecting plant and animal life, after dilution it is not practically possible to store them properly. Different scientists all over the world and particularly from China working day and night to develop a protocol for utilization of red mud waste or detoxify red mud waste or reduce the impact of waste either by addition or by deletion of chemicals, using passivators for reclamation, developing new protocols for physico-chemical treatment for red mud wastes, biological treatment protocols for treatment of waste, developing bioremediation strategies for red mud waste treatment, or growing select plants which can grow with red mud wastes without any significant impact, or resistant select crop plants to grow and produce products of human interest, or use chemicals which will not harm the crop plant but can influence the red mud waste and render the red mud waste impact less or remove the red mud waste dried cakes and transport elsewhere for construction under ground filling or construction of roads (bottom filling) and cover them with normal soil on top, reduce exposure of red mud waste to atmosphere and climatic conditions particularly rain? With advancement of Science and Technology, new protocols and strategies need to be formulated and developed for red mud waste treatment, clean waste disposal, recycling technology to be developed or the Bayer's process of extraction of alumina needs to be replaced by a simpler technique or a new technique which will not produce these types of toxic wastes harmful for all plants and animals and ultimately human life.

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Declarations:

Author Contribution statement

Prof. A.K. Panigrahi: Conceptualization, planning, supervision, field visit, script preparation, reviewing and editing. Dr. A. Leelaveni- supervision and 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.

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