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ECO-PHYSIOLOGICAL EFFECTS OF RED MUD WASTE LECHATE OF A ALUMINA INDUSTRY ON A CROP DURING SEED GERMINATION & SEEDLING GROWTH UNDER LABORATORY CONTROLLED CONDITIONS

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ABSTRACT

The industry NALCO situated at Damonjodi, Koraput produces good quality Alumina and discharges the red mud waste into a red mud pond located nearby. The lechate coming out from the red mud pond contaminates the surrounding crop fields. The present study aims at understanding the impact of the lechate of RMP on a crop seed during germination and seedling growth under laboratory controlled conditions in pot culture experiments. The impact of RMW Extract was severe indicating toxic nature of the waste. After application of lechate waste germination of seeds declined significantly. The pigments like chlorophyll, pheophytin and carotenoid content significantly depleted in pot culture. The rate of photosynthesis, rate of respiration and GPP values decreased significantly in RMWE exposed seedlings. The total ATPase activity also decreased effectively indicating the impact of the toxicant on the seed biology activities of RMW Extract exposed seedlings when compared to control seed biology parameters. The roots and shoots of the seedlings accumulated significant amount of alumina in their tissues. The observed effects were only due to alumina absorption and retention along with other soluble chemicals present in the red mud waste extract.

INTRODUCTION

At present, the industry is the focus of attention, the world-over, as strong polluter of the environment. Chemical industry in India has grown up phenomenally since independence. There is to-day about 4000 chemical factories in India. They release large quantities of chemicals in the form of gas, liquid 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. The present study site is located at the contaminated site near alumina industry at Damonjodi. It was reported (Panda *et al*., 2017; Patnaik *et al*., 2023) that the red mud waste is deadly toxic and affects the crop plants. The red mud waste contained huge amount of heavy metals and the pH was as high as around 14 and gets diluted during rainy season. Olszewska *et al*., (2016) reported significant amount of arsenic in the red mud waste and also opined that arsenic can bioconcentrate in different trophic levels and can biomagnify in the food chain. The same authors found significant amount of arsenic in few macrophytes and also concluded that inorganic arsenic content was more than organic arsenic in macrophytes. Ghorbani *et al*., (2009) studied the biological leaching of alumina from red mud bauxite waste by isolated fungi under laboratory conditions. 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

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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. During field study at the contaminated site, we observed the impact of red mud waste in the crop fields. No rice is cultivated in that area and farmers suffered a lot. We planned to provide an alternate crop for cultivation. The present study was designed to understand the impact of red mud waste on different crop seeds. After careful selection, we found the green gram seeds showed promise. The present studies aims at understanding the impact of the waste during germination of seed and establishment of seedlings till fruiting time and suggest a possible range of concentrations of the red mud waste for the farmers.

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.

NALCO produces calcined allumina at refinery complex with the specifications as mentioned .by Panda *et al*., 2017 and Patnaik et al, 2023).

MATERIALS AND METHODS

Toxicant: Red mud waste extract: The extract is prepared as explained by Patnaik *et al*., (2023) in the laboratory and used for the pot culture experiments.

Red mud waste extract preparation: 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 of the waste, which is used for e experiments. This is the red mud extract RME or RMWE and known as the test toxicant.

Test material: Green gram seeds. *Vighna radiata*,(L.) Wilczek. Pure line uncontaminated seeds of green gram were obtained

(Photo-1: Google maps showing the satellite view of in and around damonjodi, nalco- industry, location, red mud pond, rmw lechate; photo-2: Photogrpah of nalco)

(Photo-3 & 4: RMP & RM Pond link area showing wastes and loss of biodiversity) from Pulses and Millet Research Station, OUAT, Ratanpur, Ganjam. Pot culture experiments were conducted at sub-lethal concentrations and LC_{10} dose of the red mud waste extract under laboratory conditions.

The amount of total chlorophyll and total pheophytin was calculated by using the formula given by Vernon (1960). The amount of carotenoid content 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). Activity of ATPase was determined in leaf tissues by measuring the amount of inorganic phosphate produced when adenosine tri-phosphate was converted to adenosine diphoshphate. Total ATPase activity was measured by estimating inorganic phosphate liberated by the method of Fiske and Subbarow (1925) as modified by Martinek (1970). Protein was determined by the procedure developed by Lowry *et al*. (1951), using a UV-visible Spectrophotometer. The ATPase activity was expressed as µmoles of inorganic phosphate (ip) liberated mg^{-1} of protein h^{-1}. All the obtained data were statistically analyzed. The amount of alumina was estimated with the help of Atomic absorption spectrophotometer. The obtained data was statistically analyzed.

RESULTS

Control seeds and seedlings looked healthy and showed better growth when compared to exposed seeds and seedlings during the whole period of experimentation. The observed results are interesting from toxicological point of view. 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 and established.. 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, the percent of seed germination increased showing a positive correlation in the control set and with the incre3ase in RMWE (toxicant) concentration, it showed a negative correlation. Hundred percent seedlings established in the control set and at sub-lethal concentrations of red mud waste extract, with the increase in RMWE concentration, the percentage of seedling establishment decreased showing a negative correlation ($r=0.955$; $p\leq 0.01$). 100% seedling establishment was noted at sub-lethal RMWE concentration; 90% seedling establishment was recorded in 1.25% toxicant concentration and 50% seedling establishment was recorded at 2.0% toxicant concentration (Fig.1). No seedling establishment was noted beyond 2.8% RMWE prepared from red mud waste collected from the red mud pond.

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.

Fig. 1. Changes in seed germination & seedling establishment of green gram seeds at different concentrations &at different time periods in pot culture.

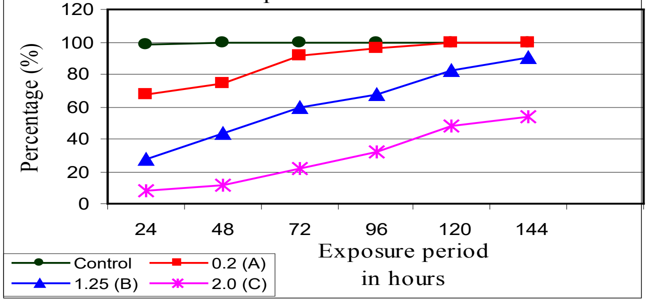
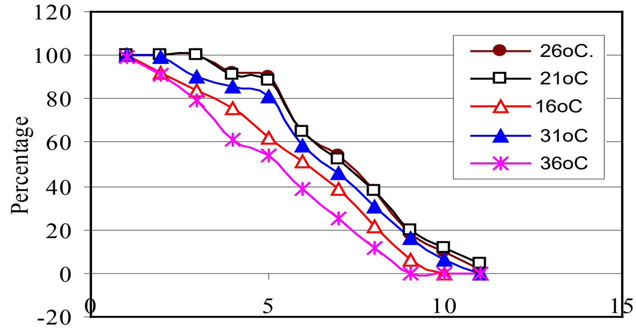
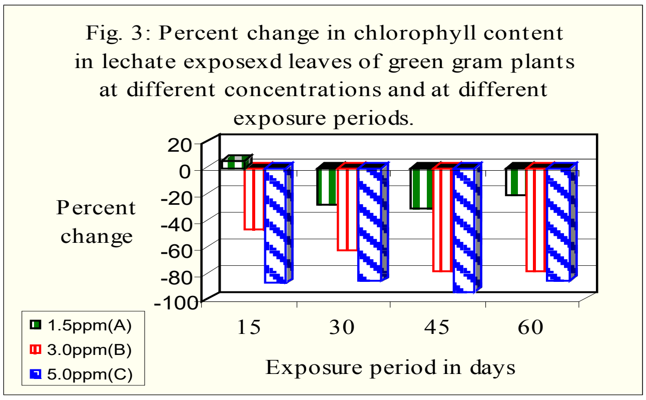
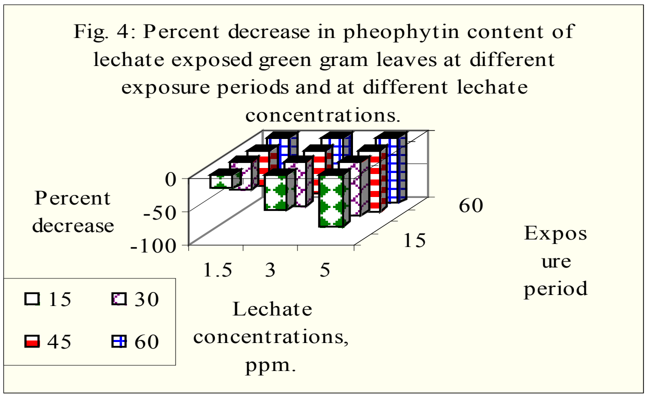


Fig. 2: Effect of temperature stress on seed germination in pot culture.



This temperature stress experiment was conducted for the first time in our laboratory under controlled conditions in a seed germinator. 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 (Fig.2). 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.2). 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.2). 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. 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. 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.2). 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 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. Our observed data and the observed trend in this piece of investigation agree with the findings of above authors. As the experiment related to temperature stress in toxic environments are lacking, our claim of reporting for the first time stands valid. This work is a pioneer work on temperature stress on red mud waste extract and impact study on crop plant. Hence care should be taken to restrict the leakage of lechate chemicals in summer months, where the temperature generally shoots up to 36 to 38°C at the discharge site during summer days. From the toxicity test, it was found that all the seeds germinated (100%) up to 0.2% (MAC- Maximum allowable concentration) 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 green gram 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 green gram seed germination increased showing a positive correlation and with toxicant concentration, it showed a negative correlation. Hundred percent green gram 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. 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. Control seeds and seedlings looked healthy and showed better growth when compared to exposed seeds and seedlings during the whole period of experimentation. The percent inhibition in seed germination increased with the increase in lechate concentration and decreased with the increase in exposure period.





It was observed that the root tip of the lechate exposed seedlings turned brown and tend 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. The chlorophyll content, pheophytin content and carotene content decreased significantly with the increase in RMWE concentration in exposed seedlings when compared to control seedlings in pot culture (Fig.3-5). The RMWE 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±0.16mg/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 (Fig.3). 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±0.02mg/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±0.44mg/g fresh weight to 0.08±0.02mg/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 (Fig.3). 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 pheophytin 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 total pheophytin content declined from 1.82±0.27mg/g fresh weight to 1.74±0.38mg/g fresh weight conc.-A, from 1.82±0.27mg/g fresh weight to 0.91±0.29mg/g fresh weight at conc.-B, and from 1.82±0.27mg/g fresh weight to 0.52±0.18mg/g fresh weight at conc.-C on 15th day of exposure (Fig.4). With the increase in toxicant concentration, the pheophytin 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 pheophytin content decreased from 1.86±0.28mg/g fresh weight to 1.31±0.47mg/g fresh weight at conc.-A, from 1.86±0.28mg/g fresh weight to 0.76±0.38mg/g fresh weight at conc.-B, and from 1.86±0.28mg/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 (Fig. 4). The total pheophytin content decreased from 2.12±0.54mg/g fresh weight to 1.42±0.51mg/g fresh weight at conc.-A, from 2.12±0.54mg/g fresh weight to 0.54±0.28mg/g fresh weight at conc.-B and from 2.12±0.54mg/g fresh weight to 0.28±0.08mg/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 pheophytin content decreased from 2.72±0.82mg/g fresh weight to 1.14±0.22mg/g fresh weight at conc.-, from2.72±0.82mg/g fresh weight to 0.31±0.07mg/g fresh weight at conc. B and from 2.72±0.82mg/g fresh weight to 0.11±0.02mg/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 (Fig. 4). With the increase in toxicant concentration and day of exposure, the pheophytin content decreased showing a negative and significant correlation. With the increase in exposure period and toxicant concentration, the percent decrease in pheophytin content showing the existence of positive correlation. Both the assessments indicated the toxicant stress. The changes in carotene content in control and leached chemicals of the red mud waste exposed green gram plant leaves at different exposure periods and at different toxicant concentrations. The carotene content declined from 0.0148±0.0016mg/g fresh weight to 0.0088±0.0016mg/g fresh weight at 15th day of exposure, where 40.54% decrease, when compared to control was recorded (Fig. 5). On 30th day of exposure, 1.78% increase in carotene content over the control value was recorded at concentration A and 45.9 % decrease was recorded at conc.-C. On 45th day of exposure, 5.8% increase in carotene content over control value was recorded in concentration A. However, 59.2% decrease was recorded in conc.-C, in contrast to conc.-A. On 60th day of exposure in conc.-A, 6.1 % increase in carotene content was recorded over the control value and in contrast, 58% and 82.3% decrease in carotene content over the control value was recorded on concentration B and conc.-C (Fig. 5), respectively. At lower concentration of the toxicant, increase in carotene level and at higher concentrations decrease in carotene level was marked at all exposure periods.

Fig. 5: Changes in carotenoid content of the leaves of green gram plants at different concentrations of lechate of RMW and exposure periods in pot culture.

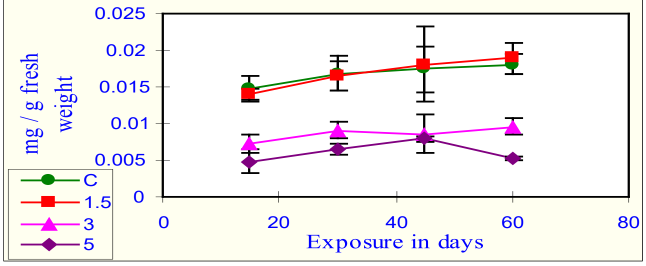
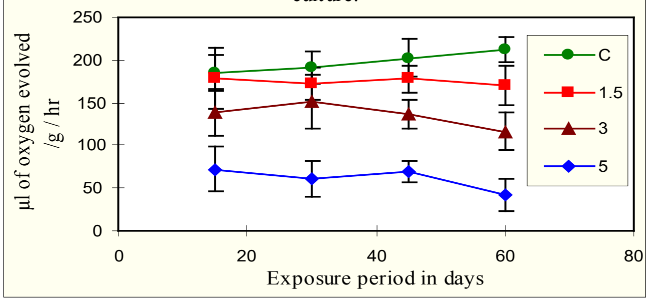


Fig. 6: Changes in rate of net photosynthesis in control and lechate exposed green gram plant leaves in pot culture.



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 photosynthetic rate (NPP) decreased from 184.6 to 91.2ml of O_2 evolved hr^{-1} g^{-1} dry weight on 15th day of exposure, where a maximum of 50.59 % decrease in NPP value was recorded. On 30th day of exposure, the NPP value depleted by 56.9 %, on 45th day of exposure, the NPP value depleted to 64.62 % and on 60th day of exposure, the NPP value depleted from 212.6 to 48.5 ml of O_2 evolved hr^{-1} g^{-1} dry weight of the seedling at concentration C, where a maximum of 77.19 % decrease in NPP value was recorded (Fig.6). Fig. 7 indicated the changes in respiration rate values in green gram exposed plant leaves at different exposure periods in pot culture. The respiration rate increased from 142.3 to 144.6ml of CO_2 evolved hr^{-1} g^{-1} dry weight in conc. A; from 142.3 to 114.2ml of CO_2 evolved hr^{-1} g^{-1} dry weight conc. B and from 142.3 to 82.4ml of CO_2 evolved hr^{-1} g^{-1} dry weight at conc. C, on 15th day of exposure, where a maximum of 42.09% decrease was recorded in conc. C. The respiration rate on 30th day of exposure declined from 151.4 to 42.7ml of CO_2 evolved hr^{-1} g^{-1} dry weight at conc. C, where a maximum of 71.8% decrease was recorded at conc. C and on 30th day of exposure. On 45th day of exposure, the respiration rate of the exposed seedlings depleted from 158.6 to 21.6ml of CO_2 evolved hr^{-1} g^{-1} dry weight at conc. C, showing a maximum depletion by 86.38% in pot culture experiment. On 60th day of exposure, the respiration rate significantly declined from 161.2 to 8.4ml of CO_2 evolved hr^{-1} g^{-1} dry weight at conc. C in pot culture, where a maximum of 94.79% decrease was recorded (Fig.7).

Fig. 7: Changes in respiration rate in control and lechate exposed green gram plants in pot culture at different exposure periods.

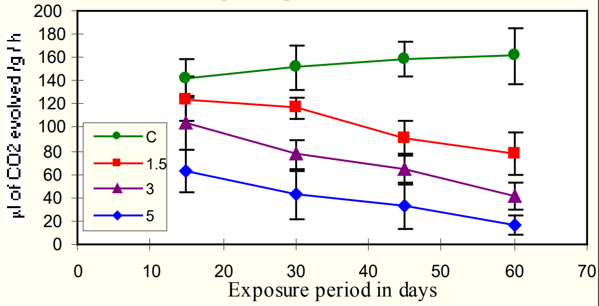
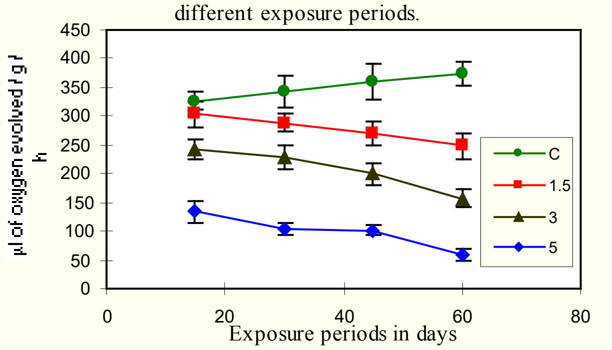


Fig. 8.Changes in gross primary productivity in control and lechate exposed green gram plants in pot culture at different exposure periods.



The gross primary productivity of the exposed seedlings drastically reduced, when compared to the control value. The GPP value decreased from 326.9 to 173.6ml of O_2 evolved hr^{-1} g^{-1} dry weight at conc. C and on 15th day of exposure; the GPP value depleted from 342.6 to 125.1ml of O_2 evolved hr^{-1} g^{-1} dry weight at conc. C and on 30th day of exposure; the GPP value depleted from 361.0 to 93.2ml of O_2 evolved hr^{-1} g^{-1} dry weight at conc. C and on 45th day of exposure and the GPP value depleted from 373.8 to 56.9 ml of O_2 evolved hr^{-1} g^{-1} dry weight at conc. C and on 60th day of exposure in pot cultures (Fig. 8). With the increase in toxicant concentration and exposure period, the GPP value significantly declined at all exposure periods. Highest depletion in GPP value was recorded in concentration C. With the increase in exposure period, the GPP value declined significantly on 15th day 46.89%; on 30th day 64.5%; on 45th day 74.2% and on 60th day 84.8% decrease was recorded, when compared to the control value. On 15th day the percent decrease was 25.7% and 46.9% in conc. B & C, respectively. On 30th day the percent decrease was 5.02%, 25.5% and 64.5% in conc.- A, B & C respectively. On 45th day, the percent decrease was 11.5%, 38.9% and 74.2% in conc.- A, B & C respectively. On 60th day, the percent decrease was 6.39%, 56.4% and 84.8% in conc.- A, B & C, respectively. The ATPase activity green gram young plant leaves decreased from 3.8±0.4mg i.p liberated/hr/100 mg dry weight to 2.8±0.6mg ip liberated/hr/100mg dry weight at conc. A; from 3.8±0.4mg ip liberated/hr/100mg dry weight to 2.1±0.5mg ip liberated/hr/100mg dry weight at conc. B and from 3.8±0.3mg i.p liberated/hr/100mg dry weight to 1.2±0.2mg ip liberated/hr/100mg dry weight in conc. C after 15days of exposure in pot culture(Fig.9). The ATPase activity green gram young plant leaves decreased from 3.9±0.4mg ip liberated/hr/100mg dry weight to 3.4±0.6mg ip liberated/hr/100mg dry weight at conc. A; from 3.8±0.3mg ip liberated/hr/100mg dry weight to 2.5±0.5mg ip liberated/hr/100mg dry weight at conc. B and from 3.8±0.3mg ip liberated/hr/100mg dry weight to 1.1±0.2mg ip liberated/hr/100 mg dry weight in conc. C after 30days of exposure in pot culture (Fig.9). The ATPase activity green gram young plant leaves decreased from 4.1±0.4mg ip liberated/hr/100mg dry weight to 2.2±0.6mg ip liberated/hr/100mg dry weight at conc. A; from 4.1± 0.4mg ip liberated/hr/100mg dry weight to 1.3±0.5mg ip liberated/hr/100mg dry weight at conc. B and from 4.1± 0.4mg ip liberated/hr/100mg dry weight to 1.3±0.2mg ip liberated/hr/100mg dry weight in conc. C after 45days of exposure in pot culture (Fig.10).

Fig. 9: Changes in ATPase activity in leaf and root of control and lechate exposed green gram plants at different exposure periods in pot culture.

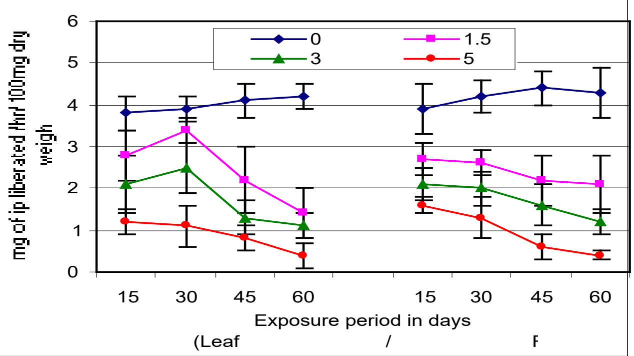
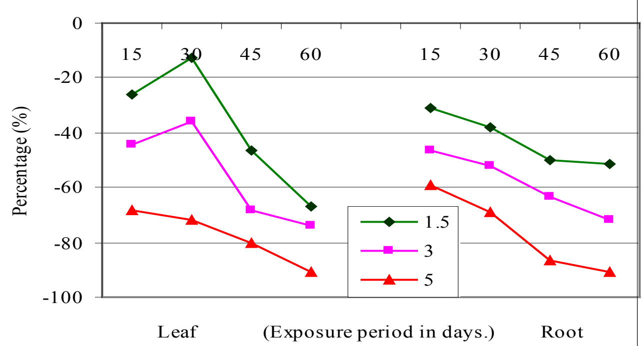
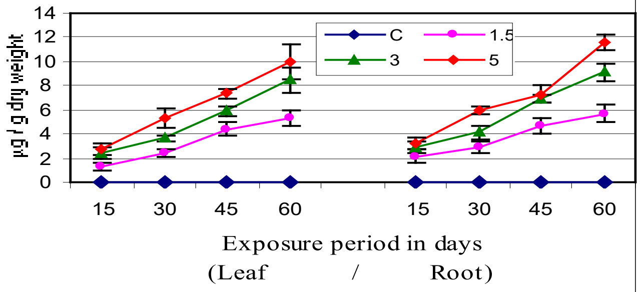


Fig.10: Percent change in ATPase activity in lechate exposed leaf and root tissues compared to control values at different exposure periods in pot culture.



The ATPase activity significantly decreased in lechate exposed green gram plant leaves at all exposure periods and at all select lechate concentrations in pot culture (Fig.45). After 15days of exposure the ATPase activity decreased by 26.3%, 44.7% and 68.4% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram leaves compared to control values. After 30days of exposure the ATPase activity decreased by 12.8%, 35.9% and 71.8% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram leaves compared to control values (Fig.45). After 45days of exposure the ATPase activity decreased by 46.3%, 68.2% and 80.5% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram leaves compared to control values. After 60days of exposure the ATPase activity decreased by 66.7%, 73.8% and 90.5% at conc. A, conc. B and conc. C respectively in lechate exposed green gram leaves compared to control values. 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. After 15days of exposure the ATPase activity decreased by 30.8%, 46.2% and 58.9% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram roots compared to control values. After 30days of exposure the ATPase activity decreased by 38.1%, 52.4% and 69.1% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram roots compared to control values (Fig.45). After 45days of exposure the ATPase activity decreased by 50%, 63.6% and 86.4% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram leaves compared to 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.10). Fig. 11 shows residual accumulation of alumina in root and leaf of green gram plants grown in pot cultures at different periods of exposure and at different lechate concentrations. No alumina was recorded in the control set green gram plant leaves. The leaves of 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. The residual accumulation of alumina increased in lechate exposed green gram plant leaves with the increase in exposure period in pot culture. 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. 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 ± 0.52 μg /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. 11). The leaf and root of control green gram plants did not show any residual accumulation of alumina indicating as standard control.

Fig. 11: Residual accumulation of alumina in leaf and root of control and lechate exposed green gram seedlings at different exposure periods in pot culture.



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 RMWE concentration. 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 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.

DISCUSSION

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 waste extract / lechate. The exposed results were significant when compared to 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. With the increase in RMWE / 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. 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. 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. 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. 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. 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% toxicant, 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.2). 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 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 findings of Radha and Panigrahi (1998) working on the industrial waste clearly indicated that these wastes are dangerously toxic and can affect rice crop and other crops. Our observed data and the observed trend in this piece of investigation agree with the findings of above authors. As the experiment related to temperature stress in toxic environments are lacking, our claim of reporting for the first time stands valid. This work is a pioneer work on temperature stress on red mud waste extract and impact study on crop plant. Hence care should be taken to restrict the leakage of lechate chemicals in summer months, where the temperature generally shoots up to 36 to 38°C at the discharge site during summer days. 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. Commercial plastics use variety of heavy metals in their formulations (Wagner *et al.,* 1992). Metallic cations are highly essential for many enzymes to achieve their catalytic activity. These cations maintain the acid base balance; and are also highly essential for the ionic composition of the body fluids. Disturbance in electrolyte concentration are quite common in toxicity. Different studies on electrolyte change resulting from toxic status have produced a variety of diverse effects, because of differential size of toxic actions in the body. Many environmental pollutants released into the atmosphere eventually settled and reach the soil by direct precipitation or deposition on vegetation and consequently as washings by rain water from vegetation to soil. The deposited chemicals on plant leaves if eaten by grazers enter into animal body and pass through the food chain. The enrichment of these toxic metals into the biosphere started with industrial pollution (Nriagu, 1970) and these metal accumulations in land and water bodies now posse's significant environmental and human health problems. These activities now require an affordable and effective solution. 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 leaves at all exposure periods and at all select lechate concentrations in pot culture. After 15days of exposure the ATPase activity decreased by 30.8%, 46.2% and 58.9% at conc. A, Conc. B and conc. C respectively in lechate exposed green gram roots compared to 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. 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.

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