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Impact Of RMP Lechate On The Physiological And Enzymological Activity Of A Crop Seed, Germinating Under Contamination Stress.

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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. Experiments were conducted at sub-lethal concentrations to study the impact of lechate waste. 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. Little insignificant increment in the photosynthetic rate was noted at sub-lethal concentration of the lechate. The ATPase enzyme activity decreased in the exposed green gram seedlings with the application of lechate compared to control green gram seedlings in petriplate culture. No increment in the enzyme activity was observed even at sub-lethal concentration of the lechate and at lower exposure period. 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 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 physiological and enzymological activity of a crop seed germinating under stress.

Key words: RMP lechate, Alumina, Pigments, RR, GPP, NPP, ATPase.

Introduction

Industry is responsible for creating a fantastic array of new chemicals every year all of which eventually find their way into the environment. For most of these chemicals, not even the chemical formulas are known and much less are known about their acute, chronic or genetic effect on the living organisms. At present, the industry is the focus of attention, the world-over, as the strong polluter of the aquatic environments. Chemical industry in India has grown up phenomenally since independence. They 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. The industry during processing of the raw materials uses many chemicals, alters physical conditions at different steps to purify the product. In the whole sequences of getting pure alumina, the industry releases many of the chemicals as gases or liquids or solids at different stages of manufacture. The 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 or in to any fresh water body can imbalance the pH of the soil or water. This high alkalinity nature of the waste makes this waste as a dangerous toxic chemical and should not be allowed to escape in to the environmental segments. 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. Reports pertaining to impact of red mud waste on toxicity and seed biological studies of crop plants are lacking. Present study aims at understanding the impact of RMP (red mud pond**)** lechate on the physiological and enzymological activity of a crop seed germinating 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.

(Google maps showing the **satellite view of in and around Damonjodi, NALCO- industry,** location, red mud pond, RMW lechate)

(Photo- Industry & RM Pond link area showing wastes and loss of biodiversity)

NALCO produces calcined allumina at refinery complex with the following specifications. Chemical properties: Typical = Al_2O_3(%)- 98.7; Na_2O(%)- 0.38 ; Fe_2O_3(%)- 0.01; SiO_2(%)- 0.012; and CaO(%)- 0.042. Alumina hydrate: Physical properties: Typical: LOI (110-1000^{o}C)%- 34-36, Moisture-3-6; Granulometry: Typical- 45Micron(%)-3-6. Chemical properties: Typical = Al_2O_3 (%) – 65 ± 0.5, Na_2O (%) Total-0.23-0.30, Na_2O (%) Soluble- 0.015-0.025, SiO_2(%)-0.007-0.010, Fe_2O_3(%)-0.006-0.008 and Hydrate Content- 99.0% (Panda *et al*., 2017).

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 petriplate culture experiments.

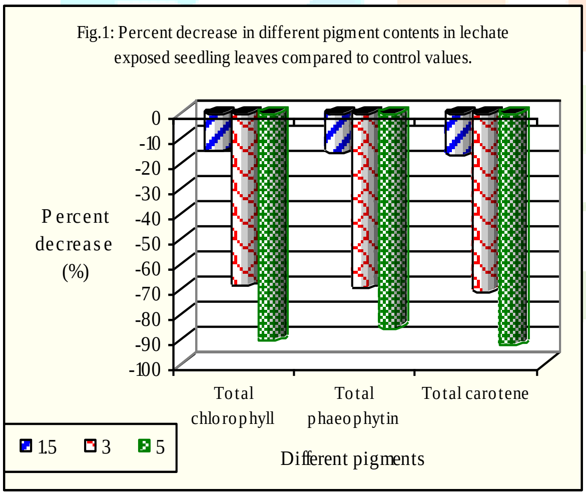
Test system: Green gram seeds (Eng. Mung, Green gram; Odia: Muga)

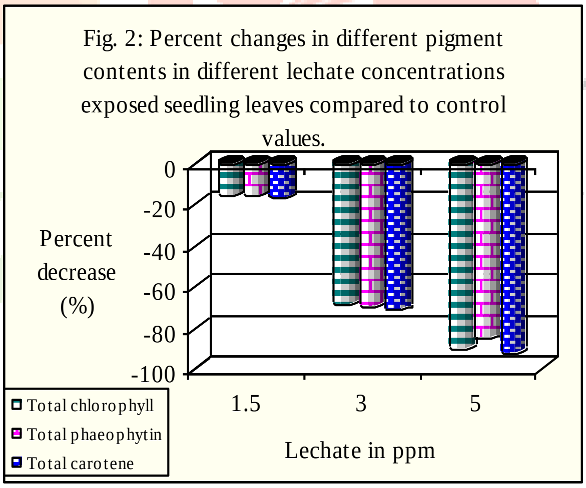
Botanical name of the crop plant: *Vighna radiata*,(L.) Wilczek. Pure line uncontaminated seeds of green gram were obtained from Pulses and Millet Research Station, OUAT, Ratanpur, Ganjam. Petriplate culture experiments were conducted at sub-lethal concentrations and LC_{10} dose of the red mud waste 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 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 di-phoshphate. Total ATPase activity was measured with Na^{+}, K^{+} and Mg^{++} in the reaction mixture. Inorganic phosphate produced was measured by the method of Fiske and Subbarow (1925) as modified by Martinek (1970). Colour development proceeded at room temperature for 30 minutes. Protein was determined by the procedure developed by Lowry *et al*. (1951), using a 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.

Results

Toxicity values obtained for green gram seed germination and seedling establishment in petriplate 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). 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±0.08 mg/g fresh weight to 0.58 ±0.11mg/g fresh weight at conc.-A, decreased from 0.56±0.08 mg/g fresh weight to 0.22±0.04 mg/g fresh weight at conc.-B, and from 0.56 ±0.08 mg/g fresh weight to 0.06±0.02mg/g fresh weight at conc. C (Fig.1). 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 96.29% decrease was recorded at conc. C of the toxicant. The total chlorophyll content increased from 1.19±0.22 mg/g fresh weight to 1.24±0.31 mg/g fresh weight at conc. A; decrease from 1.14±0.3 mg/g fresh weight to 0.48±0.2 mg/g fresh weight at conc. B and from 1.19±0.22mg/g fresh weight to 0.09±0.2 mg/g fresh weight at conc.-C after 144 hours of exposure. A maximum of 92.44% decrease in total chlorophyll content was recorded at concentration C, when compared to the control value (Fig.1). The total pheophytin 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±0.05mg/g fresh weight at conc.-B and from 0.96±0.66mg/g fresh weight to 0.08±0.01mg/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 ±0.0014mg/g fresh weight to 0.0141±0.0008mg/g fresh weight at conc.-B and from 0.0146 ± 0.0014 mg/g fresh weight to 0.0008±0.0001mg/g fresh weight at conc.-C. A maximum of 94.52% decrease over the control value was recorded at conc.-C (Fig.2).





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 analysis of variance ratio test conducted basing on the data indicated the existence of significant difference between columns and non-significant difference between rows. Interesting changes in rate of respiration, rate of photosynthesis (NPP) and Gross Primary Productivity (GPP) in control and toxicant exposed 144-hour-old seedlings in petriplate culture, grown in a seed germinator, under controlled conditions, were observed. 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 91.2µl 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 (Fig.3). On 30th day of exposure, the NPP value depleted from 191.2 to 82.4µl of O_2 evolved hr^{-1} g^{-1} dry weight at concentration C, where a maximum of 56.9 % decrease was recorded. On 45th day of exposure, the NPP value depleted from 202.4 to 71.6 µl of O_2 evolved hr^{-1} g^{-1} dry weight of the seedlings at concentration C, where a maximum of 64.62 % decrease was recorded. On 60th day of exposure, the NPP value depleted from 212.6 to 48.5 µl 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.3).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 concentration 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. The rate of photosynthesis increased from 224.8±16.4 to 246.2±28.5µl of O_2 evolved hr^{-1} g^{-1} dry weight of the seedlings in conc. A; the rate of photosynthesis decreased from 224.8±16.4 to 126.4±18.8µl of O_2 evolved hr^{-1} g^{-1} dry weight of the seedlings in conc. B and from 224.8±16.4 to 74.6±8.5µl of O_2 evolved hr^{-1} g^{-1} dry weight of the 144 hour old seedlings in petriplate cultures. The gross primary productivity decreased in exposed seedlings, when compared to control seedlings. The GPP value decreased from 461.3 to 454.6 at concentration A, from 461.3 to 229.0 and from 461.3 to 139.1µl of O_2 evolved hr^{-1} g^{-1} dry weight of the 144-hour-old seedlings in petriplate culture (Fig. 3).

Fig. 3: Percent changes in RR, PR and GPP value in lechate exposed seedlings compared to control values in green gram seedlings in petriplate culture.

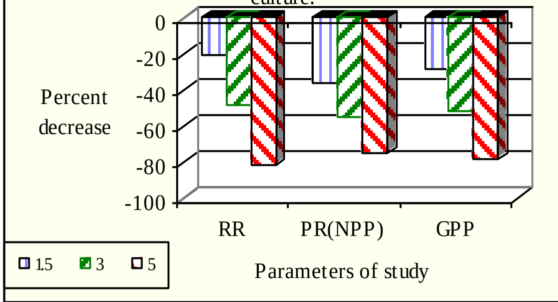


Fig. 4: Changes in ATPase activity in control and lechate exposed green gram seedlings in petriplate culture.

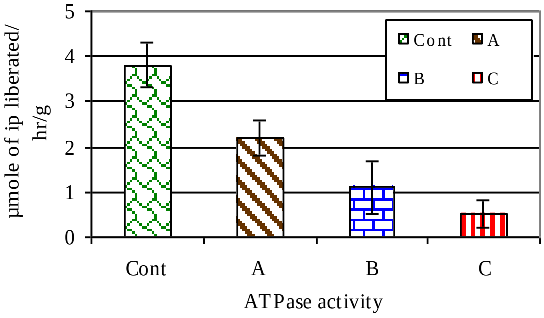


Fig. 5: Percent changes in enzyme activity in exposed shoot of lechate exposed seedlings in petriplate culture.

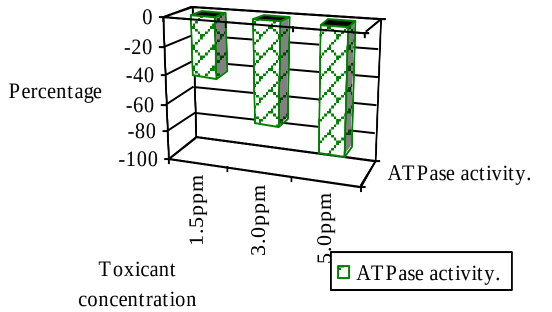
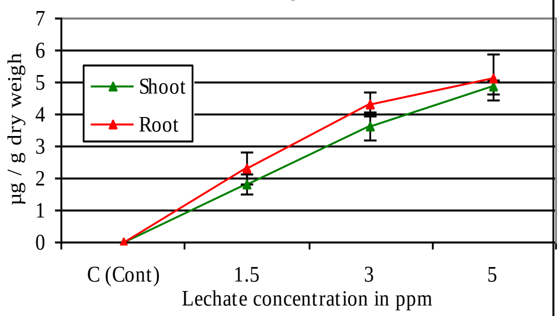


Fig. 6: Residual alumina accumulation in root and shoot of green gram seedlings in petriplate culture after 144hrs of germination.



The changes in ATPase activity of 144-hour-old green gram seedlings in different concentrations of the toxicant in Petri plate culture, grown in a seed germinator under controlled conditions showed features related to toxicity. The ATPase activity of the 144-hr-old green gram seedlings, decreased with the increase in toxicant concentration, when compared to the control value. The ATPase activity decreased from 3..8 ± 0.3mg i.p liberated / hr/ 100 mg dry weight to 2.2 ±0.6mg i.p liberated / hr/ 100 mg dry weight at conc. A; from 3.8±0.3mg i.p liberated / hr/ 100 mg dry weight to 1.1±0.5mg i.p liberated / hr/ 100 mg dry weight at conc. B and from 3.8±0.3mg i.p liberated / hr/ 100 mg dry weight to 0.5±0.2mg i.p liberated / hr/ 100 mg dry weight in conc. C. A significant ($r = -0.982, p \le 0.05$) negative correlation between the parameter with the toxicant concentration was marked. The ATPase activity decreased by 42.1% in conc. A, 71.1% in conc. B and 86.8% decrease in conc. C, when compared to the control value (Fig. 4 & 5). All the obtained values were statistically significant. ATPase activity of the 144-hour-old seedlings showed the existence of significant ($p \le 0.05$) negative correlation with the RMW lechate concentration. Fig.6 shows the changes in residual accumulation of alumina in shoot and root of 144hr old green gram seedlings in Petri plate culture exposed to different concentrations of the lechate of red mud pond waste collected from NALCO site. 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 petriplate culture (Fig.6). It was observed that the pigments, physiological parameters and enzyme activity decreased with the increase in alumina concentration in germinating seedlings.

Discussion

The red mud waste is a complex mixture of more than 87 metals and 137 compounds as reported earlier. Ghorbani *et al.*, (2009) studied the biological leaching of aluminum 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 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. 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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Conflict of interest: The authors declare that they have no conflict of interest for this publication.

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