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Biochemical Changes Impacted By A Heavy Metal Lead Nitrate On *Eleusine Coracana*, Gaertn Pot Culture Under Laboratory Controlled Conditions.

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Abstract

Experiments were conducted at 14.6mg of lead nitrate kg^{-1} dry soil mixture, as MAC dose in pot cultures. The dose was given once for short term experiments (6days), twice for 15days and thrice for 30days to maintain constancy of the medium. Biomolecular analysis (DNA, RNA, protein, FAA content) indicated that FAA and RNA content slightly increased whereas other biomolecules decreased significantly in lead nitrate exposed seedlings compared to control seedlings. Noteworthy changes in DNA content was not observed in exposed seedlings, indicating no cell death induced by lead. But the interference of lead in biomolecular synthesis can not be ruled out.DNA, RNA, protein and free amino acid content of the lead exposed seedlings and plants, significantly declined at all exposure periods and the impact was severe on 30^{th} day old ragi plants in pot culture experiments. The protein content in exposed plants significantly declined at all exposure periods when compared to control value and the impact was severe on 30^{th} day (59.3% decrease) old ragi plants. The protein content increased with the increase in exposure period in case of control set. The free amino acid content in exposed plants significantly declined at all exposure periods when compared to control value and the impact was severe on 30^{th} day old ragi plants. The free amino acid content increased with the increase in exposure period in case of control set.

Key words: Lead nitrate, Ragi, biomolecules, DNA, RNA, Protein, FAA

Introduction

The automobiles discharge a huge amount of exhausts containing poisonous gases including lead and some other chemicals (Sahu and Panigrahi, 2015, Barik and Sahu, 2017). Though lead free petrol and diesel are available in the market but due to adulteration of petroleum products used in vehicles, lead is still a part of the petroleum products. The air around the freeways and highways get contaminated with automobile exhausts and dusts suspended in the air due to vehicular traffic. These particulates, dusts, gaseous chemicals and other chemicals settle on the leaves of the plants. These deposited chemicals were surface absorbed and the pollutant enters into a plant body. Many a times we accept that the plants are good trapper of pollutants from the air and these plants act as air purifiers. The absorbed pollutants reach to the active sites of metabolism and influence all metabolic activities. Lead is a known heavy metal pollutant, affects plant survival, growth and development. Barik and Sahu (2017) reported lead availability in plants collected from a different location at NH-5, Keshpur ghat area. Significant amount of lead was reported by the same authors in perennial plants. No residual accumulation of lead was reported in young plants and herbs present in the same area (Barik and Sahu, 2017). Radha and Panigrahi (1998), Radha *et al*., 2002, 2003 and 2013 reported the impact of mercury contained solid waste discharged from a chlor-alkali industry on a crop plant and clearly indicated that the heavy metal, mercury is dangerously toxic and affects all crop plants. Bücker-Neto *et al*., (2017) reported that "heavy metals are natural non-biodegradable constituents that accumulate and persist indefinitely in the ecosystem as a result of human activities"; "since the industrial revolution, the concentration of cadmium, arsenic, lead, mercury and zinc, amongst others, have increasingly contaminated soil and water resources, leading to significant yield losses in plants" and "understanding the molecular and physiological responses of plants to heavy metal stress was critical in order to maximize their productivity".

In the review, Bücker-Neto *et al*., (2017) the authors discussed "current knowledge about the role of the plant growth hormones abscisic acid, auxin, brassinosteroid and ethylene in signaling pathways, defense mechanisms and alleviation of heavy metal toxicity". Bücker-Neto *et al*., (2017) opined that "understanding how plants can translate the signals from an ever changing environment into physiological behavior is essential for reducing harmful effects caused by abiotic stresses, such as heavy metal toxicity". At present all the three segments of the environment are seriously affected by the pollutants released from variety of sources and mostly are man made and man planned. Lead is ai important toxicant in aquatic and terrestrial ecosystems. Many countries adopted different types of regulatory measures to restrict the discharge and input of lead into the environment. Lead finds its use in many ways and interferes with human life. This is one of the reasons why lead is available in all the three environmental segments like air, water and land. Whatever the amount of lead that is available in air finally gets precipitated on soil and water body. The lead in water bodies gets precipitated to become a part of the soil. Soil is the ultimate sink for all the lead and its compounds. Day by day lead concentration is increasing in soil and likely this metal concentration may not decrease in future unless or until a specialized technique should be invented or a mechanism is to be evolved to recycle lead or removal technology of lead from the environmental segments or a very safe final disposal technology for lead. Generally lead is deposited on the surface soils and chances of quick infiltration and leaching to deeper layers is bleak. Reports indicated that lead concentrates in the bottom of soil crust and contaminates the ground water significantly. Plants mostly absorb heavy metals from the soil and accumulate in different organs of the plant body. Lead concentration is very high on soil surface and also on the surface layer of ground water. Lead concentration decreases with the increase in soil depth. Plants after absorption of lead along with water and other essential micro and macro nutrients, translocate to different tissues and organs of the body. Lead is not an essential chemical required for plant growth and development. The nutrients absorbed were used up in different metabolic functions of the plant, supporting growth and development. Lead enters into the cells and remains in the protoplasm. It is a protoplasmic poison, acts slowly and attacks different biomolecules disrupting metabolic functions and inhibits synthesis of biomolecules affecting the growth and development of plants. Ultimately lead affects the productivity of the plants. It was observed that lead concentration in soil is very high near industrial sources and also lead concentration in water bodies receiving industrial discharges is very high and significant. It was reported that the crop plants growing nearby places of an industry using lead or lead contained raw material required for the industry containing lead, accumulate lead significantly. It was also observed that the productivity of the crop plant is drastically depleted. It also affects the pigment contents of the crop plants and other aquatic and terrestrial plants. Lead has many uses in our day to day life and also in the modern day science. Lead is used as electrodes in the process of electrolysis; lead is used in the glass of computer and television screens to avoid the radiation for users; used as a coloring element in ceramic glazes as projectiles, in some candles; lead is also used in sheeting, cables, solders, lead crystal glassware, ammunitions; Lead is used as bearings and as weight in sports equipments; Lead is used in many industrial processes (Industries like Paint, fertilizer, pesticide industry to produce insecticides).

The present piece of work has been planned to find out the impact of lead nitrate on the biomolecular content of ragi, *Eleusine coracana*, Gaertn seedlings and plants at different exposure periods in pot cultures under laboratory controlled conditions to understand the lead poisoning, route of absorption and its impact on ragi seeds and seedlings and growing plant.

Materials & Methods

Test Organism: Finger millet, Ragi, Odia: Mandia

Classification: Family: Poaceae; Genus: *Eleusine;* Species: *coracana* ;

Binomial Plant Name: *Eleusine coracana*, (L.) Gaertn.

Test chemical: Lead nitrate: $Pb(NO_3)_2$

Garden soil was collected, sun dried and powdered. Dried cow dung manure was brought air dried under sun and powdered. Garden soil powder was mixed with cow dung powder at 3:1 ratio. The soil mixture was weighed and 1kg of soil mix was added to each pot. The content of each pot was sterilized with steam, allowed to cool in a sterilized culture room and kept inside culture room till use. Pot culture experiments were conducted by taking the toxicity values from toxicity studies (Barik, 2016). Experiments were conducted at sub-lethal and lethal concentration values.

Biochemical studies: The leaf of the seedlings was washed thoroughly in double distilled water. !00mg of leaf tissue was taken and homogenized with 5 ml of 80% ethanol (v/v) in a micro-tissue homogenizer to extract free amino acids (FAA). The extract was centrifuged and the supernatant was taken for estimation of amino acids (FAA) by ninhydrin method following the procedure of Lee & Takahasi (1966). The residue was washed in 5% cold Tri Chlor Acetic acid (TCA), centrifuged and further extracted in 5 ml of 10% TCA (w/v). Total DNA was measured by diphenylamine reaction method (Herbert et al., 1971) and RNA by

Orcinol reagent method of Volkin and Cohn (1954). The protein precipitated by TCA in the above residue was estimated by the procedure of Lowry *et al.* (1951). The obtained data was statistically analyzed.

Results

The changes in biomolecular contents like DNA, RNA, protein and FAA content of ragi plants exposed to different concentrations of lead nitrate like MAC, LC10 and LC50 doses at different days of exposure like 6, 15 and 30days were tested to find out the affects of the toxicant in pot culture. The petriplate studies of ragi seedlings indicated the severe effects of the toxicant on cellular biomolecules. This study may not give any conclusive evidence on the impact of the toxicant at molecular level but it can provide us information pertaining to ecological impact of the toxicant on the growth and development ragi seedlings. The changes in DNA content in control and lead nitrate exposed seedling leaves at different exposure periods and at different lead nitrate concentrations in pot culture and the percent change of this parameter has been shown in Fig.A4A. The DNA content ranged between 0.35 ± 0.09mg / g FW to 0.37 ± 0.07mg / g FW from 6th to 30th day of exposure in the control pot set. The DNA content increased at sub-lethal exposure to lead nitrate. The increase in DNA content indicated new cell formation and growth of the growing seedling. The increase in DNA content was linear and steady (Fig.A1A). The DNA content of 6th day old seedlings of pot culture increased from 0.35 ± 0.09mg / g FW to 0.36 ± 0.11mg / g FW at LC_{00} or at MAC value of lead nitrate (Fig.A1A). The DNA content of 6th day old seedlings of pot culture decreased from 0.35 ± 0.09mg / g FW to 0.31 ± 0.07mg / g FW in pot culture at LC_{10} value of lead nitrate and the DNA content of 6th day old seedlings of pot culture significantly decreased from 0.35 ± 0.09mg / g FW to 0.24 ± 0.08mg / g FW in pot culture at LC_{50} value of lead nitrate (Fig.A1A). The DNA content of 15th day old seedlings of pot culture increased from 0.37 ± 0.07mg / g FW to 0.40 ± 0.09mg / g FW at LC_{00} or at MAC value of lead nitrate. The DNA content of 15th day old seedlings of pot culture decreased from 0.37 ± 0.07mg / g FW to 0.28 ± 0.11mg / g FW in pot culture at LC_{10} value of lead nitrate and the DNA content of 15th day old seedlings of pot culture significantly decreased from 0.37 ± 0.07mg / g FW to 0.22 ± 0.07mg / g FW in pot culture at LC_{50} value of lead nitrate. The DNA content of 15th day old seedlings of pot culture increased from 0.37 ± 0.07mg / g FW to 0.40 ± 0.09mg / g FW at LC_{00} or at MAC value of lead nitrate (Fig.A1A). The DNA content of 15th day old seedlings of pot culture decreased from 0.37 ± 0.07mg / g FW to 0.28 ± 0.11mg / g FW in pot culture at LC_{10} value of lead nitrate and the DNA content of 15th day old seedlings of pot culture significantly decreased from 0.37 ± 0.07mg / g FW to 0.22 ± 0.07mg / g FW in pot culture at LC_{50} value of lead nitrate (Fig.A1A).

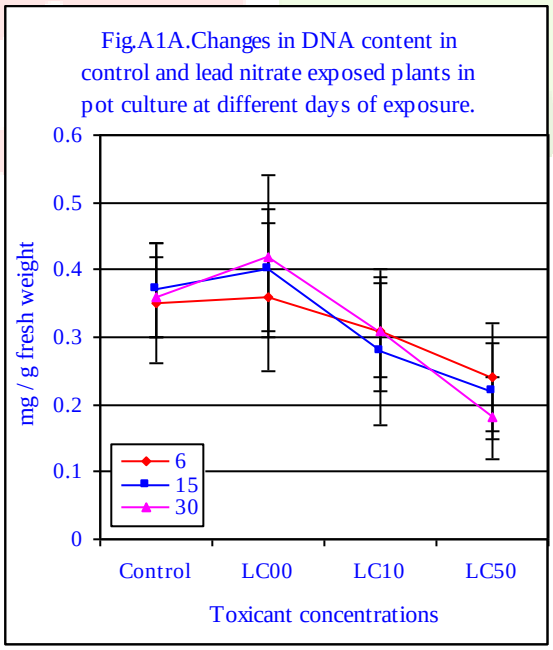


Fig.A1A.Changes in DNA content in control and lead nitrate exposed plants in pot culture at different days of exposure.

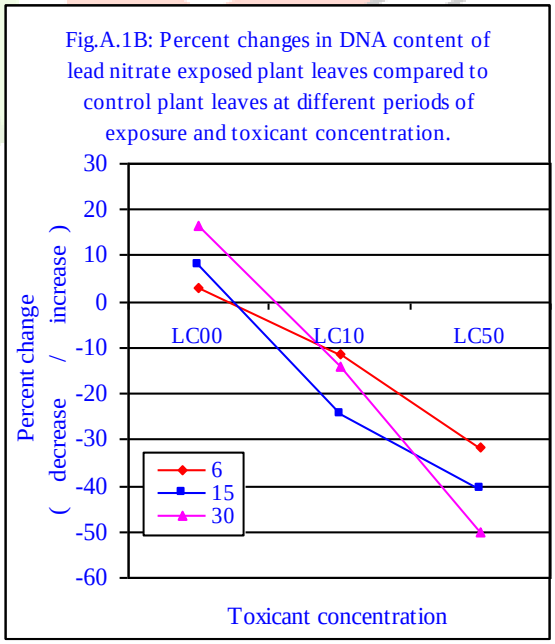
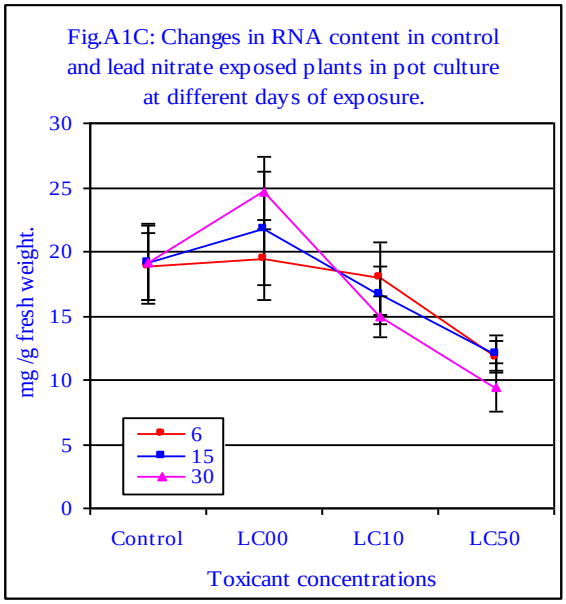
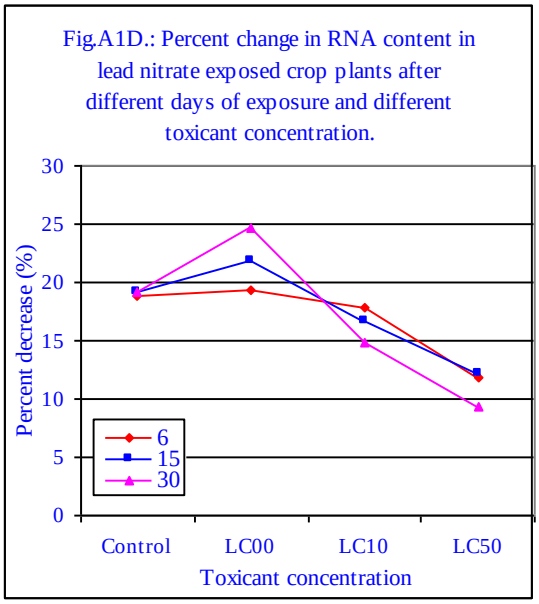
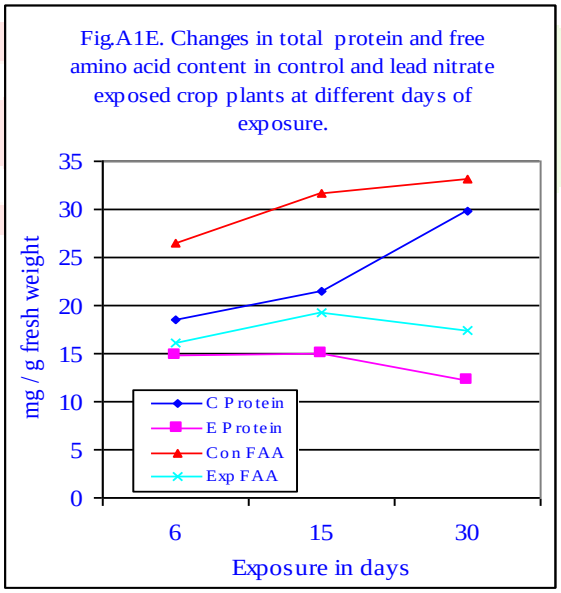


Fig.A.1B: Percent changes in DNA content of lead nitrate exposed plant leaves compared to control plant leaves at different periods of exposure and toxicant concentration.





The DNA content of 30th day old seedlings of pot culture increased from 0.36 ± 0.06mg / g FW to 0.42 ± 0.12mg / g FW at LC_{00} or at MAC value of lead nitrate. The DNA content of 30th day old seedlings of pot culture decreased from 0.36 ± 0.06mg / g FW to 0.31 ± 0.09mg / g FW in pot culture at LC_{10} value of lead nitrate and the DNA content of 30th day old seedlings of pot culture significantly decreased from 0.36 ± 0.06mg / g FW to 0.18 ± 0.06mg / g FW in pot culture at LC_{50} value of lead nitrate (Fig.A1A). The changes in RNA content in control and lead nitrate exposed seedling leaves at different exposure periods and at different lead nitrate concentrations in pot culture and the percent change of this parameter has been shown in Fig.A1C. The RNA content ranged between 18.8 ± 2.6mg / g FW to 19.2 ± 2.9mg / g FW from 6th to 30th day of exposure in the control pot set. The RNA content of exposed seedling leaves increased at sub-lethal exposure to lead nitrate. The increase in RNA content indicated increased RNA synthesis which becomes the base for new cell formation and growth of the growing seedlings at sub-lethal concentrations. The increase in RNA content was linear (Fig.A1C).



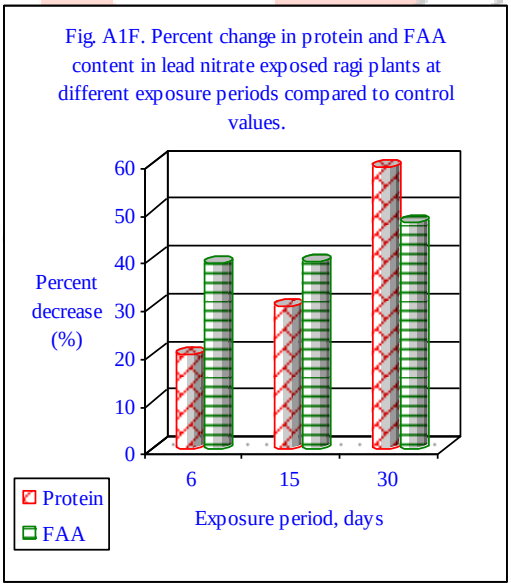
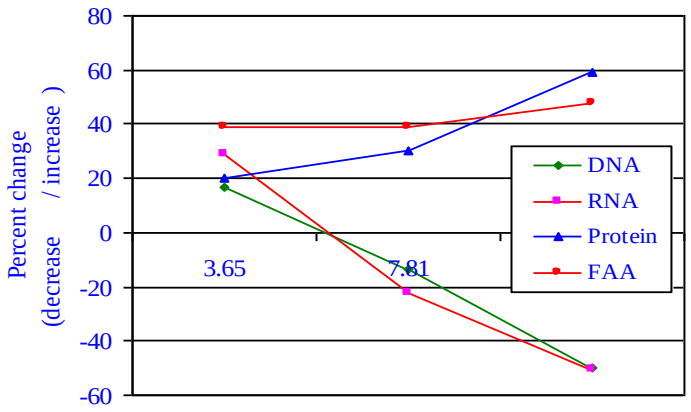


Fig. A.1G: Percent change in biomolecular content in lead nitrate exposed ragi plants in pot culture at different residual lead level.



The RNA content of 6th day old seedlings of pot culture increased from 18.8 ± 2.6mg / g FW to 19.4 ± 3.1mg / g FW at LC_{00} or at MAC value of lead nitrate. The RNA content of 6th day old seedlings of pot culture decreased from 18.8 ± 2.6mg / g FW to 17.9 ± 2.8mg / g FW in pot culture at LC_{10} value of lead nitrate and the RNA content of 6th day old seedlings of pot culture significantly decreased from 18.8 ± 2.6mg / g FW to 11.8 ± 1.2mg / g FW in pot culture at LC_{50} value of lead nitrate (Fig.A1C). The RNA content of 15th day old seedlings of pot culture increased from 19.2 ± 2.9mg / g FW to 21.8 ± 4.4mg / g FW at LC_{00} or at MAC value of lead nitrate. The RNA content of 15th day old seedlings of pot culture decreased from 19.2 ± 2.9mg / g FW to 16.6 ± 2.2mg / g FW in pot culture at LC_{10} value of lead nitrate and the RNA content of 15th day old seedlings of pot culture significantly decreased from 19.2 ± 2.9mg / g FW to 12.1 ± 1.4mg / g FW in pot culture at LC_{50} value of lead nitrate (Fig.A1C). The RNA content of 30th day old seedlings of pot culture increased from 19.1 ± 3.1mg / g FW to 24.6 ± 2.8mg / g FW at LC_{00} or at MAC value of lead nitrate (Fig.A1C). The RNA content of 15th day old seedlings of pot culture decreased from 19.1 ± 3.1mg / g FW to 14.9 ± 1.6mg / g FW in pot culture at LC_{10} value of lead nitrate and the RNA content of 15th day old seedlings of pot culture significantly decreased from 19.1 ± 3.1mg / g FW to 9.4 ± 1.9mg / g FW in pot culture at LC_{50} value of lead nitrate (Fig.A1C). The DNA content increased by 2.8%, 8.1% and 16.7% on 6th, 15th and 30th day of exposure at sub-lethal concentration (MAC value) of lead nitrate when compared to respective standard values of control. With the increase in lead nitrate dose, the DNA content declined at all exposure periods. The DNA content decreased by 11.4%, 24.3% and 13.9% at LC_{10} dose of lead nitrate compared to respective standard value. The DNA content decreased by 31.4%, 40.5% and 50% at LC_{50} dose of lead nitrate compared to their respective control value (Fig.A1B). The RNA content increased by 3.2%, 13.5% and 28.8% on 6th, 15th and 30th day of exposure at sub-lethal concentration (MAC value) of lead nitrate when compared to respective standard values of control. With the increase in lead nitrate dose, the RNA content decreased at all exposure periods. The RNA content decreased by 4.8%, 13.5% and 21.9% at LC_{10} dose of lead nitrate compared to respective standard value. The RNA content decreased by 37.2%, 36.9% and 50.8% at LC_{50} dose of lead nitrate compared to their respective control value (Fig.A1D). The Anova test for both the sets of data indicated the existence of significant differences between rows and insignificant difference between columns. The protein content significantly declined at all exposure periods and the impact was severe on 30th day old ragi plant leaves. The protein content decreased from 29.8 ± 2.88mg / g FW to 12.14 ± 0.56mg. / g FW on 30th day of exposure (Fig. A1E), showing a maximum of 59.3% decrease over the control value (Fig.A1E). The percent decrease in chlorophyll content was higher at higher exposure period showing a positive correlation ($r= 0.985$; $P\leq 0.01$). The protein content depleted by 19.95% on 6th day, 30.1% on 15th day and 59.3% on 30th day of exposure, when compared to respective control values (Fig. A1E).Fig.A1E showed the changes in free amino acid content of control and lead nitrate exposed leaves at different days of exposure in pot culture. The free amino acid content significantly declined at all exposure periods and the impact was severe on 30th day old ragi plant leaves. The free amino acid content decreased from 33.2±2.4mg.g^{-1} FW to 17.4±2.9mg.g^{-1} FW on 30th day of exposure (Fig. A1F), showing a maximum of 47.6% decrease over the control value indicating a positive correlation ($r= 0.976$; $P\leq 0.05$) with the increase in exposure period. The free amino acid content depleted by 39.02% on 6th day, 39.24% on 15th day and 47.59% on 30th day of exposure, when compared to respective control values (Fig.A1G). It was observed that the protein content decreased significantly indicating proteolysis induced by lead nitrate and increase in FAA content in the exposed seedling leaves might indicate non condensation of

amino acids into proteins or more free amino acids because of proteolysis induced by lead nitrate in the exposed ragi plantations.

Discussion

Lead is a very well known heavy metal pollutant affects animal and plant survival, growth and development. Barik and Sahu (2017) reported lead availability in plants collected from different locations at NH-5 (now NH-16), Keshpur Ghat area and NH-157, Kalinga Ghat area. Significant amount of lead was reported by the same authors in perennial plants. Effect of lead on algae has been reported by Choudhury (2016). Wide ranges of pollutants generally target the plants and the impact varies and is dependent on the concentration, speciation and accordingly the toxicity will change. These pollutants mostly find their way in to the plant body from the soil (Arshad *et al*., 2008) or from the air sources where atmosphere is the sink after discharge / leaching (Barik *et al.*, 2017, 2018a). Lead is considered to the most poisonous and frequently encountered pollutant affecting the live organisms and plants in particular (Shahid *et al*., 2011 and Grover *et al*., 2010). In the past we have used lead in many ways without knowing its toxic affects after industrial revolution. Once we knew the hazardous effects leading to disease and death, either we banned its use or decreased the use or no longer have we used the metal for activity. Under forced circumstances when a good substitute is not available at some places still we find the use of lead like lead batteries. Of course now substitutes are available and slowly we will phase out lead and we will not depend on lead. We have already experienced the bad effects of lead on plants, animals and human beings all over the world. US EPA (1986) report indicated that "lead accumulates in the soil when the soil is rich in organic compounds. The organic matter present in the top layer of soil could retain significant quantity of atmospheric lead from the atmosphere, where lead remains as dust and particles. This lead gets mixed with soil chemicals and they are absorbed by the microorganisms, plants and grazing food chain. US EPA (1986) suggested that "the uneven distribution of lead in the ecosystems can displace other metals from the binding sites on the organic matter. It may hinder the chemical breakdown of inorganic soil fragments and lead in the soil may become more soluble, thus being more readily available to be taken up by plants'. Plants on land tend to absorb lead from the soil and retain most of this in their roots. There is some evidence that plant foliage may also take up lead (and it is possible that this lead is moved to other parts of the plant)". It was reported that "the absorption by roots, toxicity and impact of lead can be reduced by the application of calcium and phosphorus in the lead contaminated soils" (UNEP, 2000). The deposition lead dust and automobile gaseous exhausts mixed with lead and atmospheric moisture forms a coating over the leaf surfaces closing the stomata and pores present in the leaves and plant parts. This coating stops gaseous exchange across stomata and the pores there by reducing the photosynthetic activity. This coating on the surface of the leaves also reduces the penetration of sunlight reducing photosynthetic activity by chlorophylls. This disruption reduces the plant gross production and induces stunting growth, induces aging, decreases photosynthetic rate, may inhibit respiration rate ultimately impacting the physiological and biochemical functions leading to death of the plants in a long run. Lead being a heavy metal is toxic and can accumulate in plant cells, tissues, parts, organs and in agricultural products (Burzynski, 1987b; Mahmoud and El-Beltagy, 1998), and on a sequence can enter human food chain (Wagner, 1993) and very difficult to control. Salt *et al*., (1998) reported that "environmental contamination with lead has accelerated due to its close relationship to industrialization and its wide usage in variety of industries including paint industry and gasoline installations". Johnson and Eaton (1980) reported that "soils contaminated with lead caused significant depletion in crop productivity thereby posing a serious problem for agriculture". Patra *et al*., (2004) indicated that "although lead is not an essential nutrient for plants, majority of lead is easily taken up by plants from the soil and accumulated in root while only a small fraction was translocated upward to the shoots". Ghani (2010) reported that "lead affects several metabolic activities in different cell compartments. The effect of lead depends on concentration, type of soil, soil properties and plant species". Munzuroglu and Geckil (2002) reported that "lead toxicity reduces seed germination percentage, affects root and shoot length, weight both fresh and dry, depletes dry matter production"; significantly decreases cell division (Eun *et al*., 2000) and severely impacts mineral nutrition (Paivoke, 2002). Mostly the lead metal ions get deposited primarily in roots after absorption because these ions first reach the roots than by transportation and translocation to different parts of the plants and shoot. Patra *et al*., (2004) reported that "in maize crops lead metal ions mostly accumulate in their roots and shoots as residual accumulation (Reddy *et al*., 2005) & interestingly the fresh plant parts are neither eaten as food nor dry parts are eaten by grazers". It seems from the report that the maize plants can be grown in lead contaminated soils to prevent entry of lead into the food chain. Whereas, it was observed that most of the crops like rice, green gram, black gram, some vegetables accumulate lead and help in trophic level transfer of the heavy metal in food chain, impacting the ecosystem functioning. Hence, it is important to find out plants which can absorb this heavy metal and retain the metal either in roots or shoots but not in seed/grain/ fruiting structures, so that these plants can be used as phytoremediation. The present effort is a step in this direction

to find out whether ragi plant can be used as a soil reclamator in lead contaminated soils and whether this plant can effectively absorb and remove significant quantity of lead from the lead contaminated soils of the contaminated sites or not? The whole story depends on the soil type, soil quality, rain fall, temperature and irrigation facility, nutrient status of the soil, suitability of the crop plant for plantation etc. because these parameters decide survivability of the plants, plant species, as the impact of these parameters will vary from species to species. Wu *et al*., (2008) reported "non availability of literature or limited availability of literature to show the link between lead toxicity and photochemical activity of PSII". Nas and Ali, (2018) reviewed "the effects of lead ion on the growth and some biochemical parameters in plants. Lead toxicity caused inhibition of ATP production, lipid peroxidation, and DNA damage by over production of ROS. In addition, lead strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and water and protein content." Nas and Ali (2018) reported that "the lead uptake is mainly regulated by PH, particle size, and cation exchange capacity of the soil, root exudation and by different other physical and chemical parameters. The high concentration of the heavy metals such as lead can cause a number of toxic symptoms in plants that may be retardation in growth (Stunted growth), negative effects on photosynthesis (chlorosis), blackening of roots and different other symptoms (Lead has the ability to inhibit photosynthesis, disturb mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability".

Lead is known to cause delay in seed germination (Barik and Sahu, 2017; Barik and Sahu, 2018a, b; Reddy *et al*., 2005), cause chlorosis of leaf (Wierzbicka, 1994) depletion in root and shoot length, root is highly impacted by lead than the shoots, as root is the primary structure coming in direct contact with the metal in soil or petriplate medium or pot soil (Barik and Sahu, 2017; Barik and Sahu, 2018a, b). Lead is also known to activate or deactivate enzymes impacting badly on plant metabolism (Van Assche and Clijsters, 1990 and Fargasová, 1994). Reports of different authors indicated that heavy metal like lead can be absorbed and translocated to different parts of the plant body. Many also reported that the accumulation was higher in roots, when compared to foliar parts. It is obvious that root is the primary site of absorption and maximum accumulation is expected to be in the roots. Some authors reported that highest accumulation was in the Brassicaceae family, *Zea mays, Pistia stratiotes* (Kumar *et al*., 1995, Małkowski *et al*., 2002, Vesely *et al*., 2012). The inference of above workers can be best used in phytoremediation process and rhizofiltration being a cost effective subset technique can be utilized in reclamation studies (Fahr *et al*., 2013 & Dushenkov *et al*., 1995). From our present study, it was observed that the impact of lead on the ragi seed biology parameters in pot culture was more acute and intense. Lead induced significant depletion in seedling growth measured by root and shoot length and seedling biomass when compared to control seedling parameters. When silicon was introduced along with lead into petriplate and pot culture, the impact of lead was not that serious when compared to singular application of lead nitrate into the growing medium, where ragi seeds were allowed to germinate and grow. The least impact or no impact might be due to silicon, which might have acted as a masking agent or inhibitory agent for lead. Most interestingly, we found that the impact of lead was more severe in petriplate culture when compared to pot culture. The ragi seeds resisted higher concentrations of lead nitrate in pot culture when compared to petriplate culture. All other environmental variables were kept constant and those seeds were grown in culture racks. Pot culture experiments were also conducted in culture rack to provide similar environment. It was observed that the minimum concentration at which seed biology of ragi seeds were inhibited in petriplate culture, at the same concentration lead nitrate did not show any impact. Impact lead was observed at higher concentrations in pot culture. This was probably due to the synergistic or antagonistic effect soil and fertilizer mixture supplied in pot culture. The soil mix in pot culture might have reduced the impact of lead on germination of seed and growth of seedling and finally seedling establishment when compared to petriplate culture. The results of the investigation showed by Bharwana *et al*., (2013) gains importance as informed that silicon played a notable role and increased the lead-mediated decrease in growth, biomass and photosynthetic parameters in cotton plants. We agree with the above authors. In our present investigation, we also observed the severe impact lead nitrate on the root and shoot growth by length, dry weight, biomass, biomolecule changes and photosynthetic efficiency in case of ragi. To their knowledge, it was claimed to be the first ever investigation on the interaction of lead and silicon in plants but is really not. Many others also conducted experiments on this issue. Much work has been done on impact lead on BGA and crop plants and possible phytoremediation by environmental chemicals on different crop plants. The results of Bharwana *et al*., (2013) also showed depleted soluble protein in both roots and leaves of cotton plants that were exposed to lead. They opined that the aforesaid effects might be because of oxidative damage which have occurred that probably decreased the protein contents and this observation was earlier reported by Nwugol and Huerta (2008). It was observed that protein content and FAA content increased even when the residual lead concentration was high. It can be attributed that both protein and FAA content increase has a relation with residual lead accumulation. The decrease in DNA and RNA content in

lead nitrate exposed seedlings was probably due to high accumulation of residual lead leading to either cell death or disruption in DNA biosynthesis and inhibition of RNA synthesis. The above statement needs further confirmation by studying other related parameters linked to DNA and RNA biosynthesis. From the observations and results, it is clear that lead nitrate is deadly toxic and can significantly affect any plant or animal. The metal can be absorbed, translocated to different parts of the body and it can accumulated in different parts of the body. It is also a fact that this chemical is not gentle or inert, it can take part in identified biochemical reactions, can become a part of physiological processes by way reacting with substrates or enzymes responsible for different activities. Thereby inhibiting a reaction or forming a complex with a suitable biomolecules making it non functional in a biological system, this can be a plant or an animal or a human being.

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