



Eco-Toxicological Impact Of Atmospheric Lead On Road Side Plants & Experimental Study On Ragi Seeds Under Laboratory Controlled Conditions.

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Highlights:

- The air in and around the National Highways & freeways are highly polluted areas polluted by the automobile exhausts.
- Plants either planted or naturally grow on roadside suffer the most because of automobile exhaust. The leaves of the plants of the contaminated area are heavily coated with dust and polluted air particulates.
- Significant reduction in plant pigment content was observed in air pollutant exposed plants of the Ghat area.
- The plants collected from the National Highway roadside contained significant amount of lead in their tissues. The impact of air pollutants were severe on plants collected from the Ghat areas.
- Experimental study in the laboratory on ragi seed indicated the acute toxic nature of lead and its impact on macromolecular content of the lead exposed seedlings.

Abstract

Field visit to Ghat areas indicated that the leaves of roadside plants were heavily coated with lead and air pollutants coming out from automobile exhausts. During rainy season the washings of the plant flow to low land areas, where different crops are cultivated. The production in these contaminated crop fields significantly reduced. Significant amount of residual lead was observed in the older plant leaves. The roadside plants were showing stunted growth and decrease in pigment content was noted in the roadside plant leaves compared to standard control plant leaves. Biomolecular analysis indicated that FAA and RNA content slightly increased whereas other biomolecules like DNA & RNA 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.

Keywords: National Highway, Air pollutants, Lead, Plants, Crop plant, Mustard seed, Seed Biology

Introduction

Lead is a very well known heavy metal pollutant affects animal and plant survival, growth and development. 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). Johnson and Eaton (1980) reported that "soils contaminated with lead caused significant depletion in crop productivity thereby posing a serious problem for agriculture". 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". Literature and information pertaining to residual lead in plants of the roadside plantations of National Highways and freeways of Odisha state is lacking and impact of lead and its compounds on crop plants is also scanty. An attempt was made to collect samples from both the roadsides of National highways and freeways passing through Kalinga Ghat area grouped under Ghumsar Forest division, Kandhamal, Odisha. During field study, it was also observed that rice, green gram, black gram and other vegetable were not grown in lead contaminated areas particularly on both the road sides of freeways and Highways because of automobile exhausts & dusts and its consequent deposit on the road side cultivated crop fields. Ragi was selected as the test material as this crop is used for cultivation by most of the farmers of the tribal area and ragi is their staple food. An experimental study was planned to assess the impact of lead on the seed biology, seedling physiology, growth and behavior of ragi seeds, seedlings and grown up plants and most important is lead accumulation in plants at different exposure periods.

Material and Methods

Field collection and study was conducted at th Ghat area of Kandhamal district. Hand sampling was done. Leaf and soil samples were collected and brought to the laboratory in ice containers for analysis. The leaves collected from both the sites, were hand washed carefully with distilled water to remove adhered dust and particulate matters on the leaf surfaces. The leaves were cleaned with soaked filter paper to remove adhered water. The pigment contents of the leaves were estimated and calculated following the method described by Vernon (1960) and Davies (1976). For residual lead analysis, known amount of leaf sample was taken in a Klein's apparatus (digester) with acid digestion mixture (Conc. Sulphuric acid and Conc. Nitric acid, 1:1 ratio) and the sample was digested till the whole sample was well digested (Wantorp and Dyfverman (1955). Minimum two cycles were maintained for all samples. After digestion, the digested extract was cooled to room temperature. After cooling, the lead content of the digested extract was estimated by using Atomic Absorption Spectrophotometer (Perkin Elmer, 3100) following the procedure of Yoshida *et al.* (1976). Estimation of amino acids (FAA) was carried out by ninhydrin method following the procedure of Lee & Takahasi (1966). 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 was estimated by the procedure of Lowry *et al.* (1951). The data was statistically analyzed to find out the levels of significance.

Results

The National Highways passing through Ganjam & Kandhamal district was selected for survey. On both the roadside terrestrial plantations and different types of crop plants cultivated by the farmers were surveyed. Our discussion with farmers revealed that rice, green gram and black gram cultivation was failure and expected production in all those crops was not possible. Those farmers are unable to grow any other vegetable or crop due to water shortage in the area. They were growing only one crop per year and hundred percent they were rain dependent. One of the reasons for failure of crop was heavy deposits of dust on the leaves of the plants due to heavy traffic because of Highway vehicular traffic. Observation of roadside plantation revealed heavy deposit of dust and aerosols on the leaf surfaces reducing sunlight penetration and depletion of gaseous exchange due to deposits on stomata. Washing of the dark grayish leaves could remove the adhered dust but oil / aerosol coating was still there indicating non removal of coating even in rainy season. Hence it was planned to test the road side plant leaves regarding the impact of this dust coating on the pigment content of the leaves and possible accumulation of heavy metals in the exposed leaves.

The plants collected from the sides of National Highways (NH) contained residual lead in their leaf tissues. Out of the samples collected, *Mangifera*, *Psidium*, *Michelia*, *Tectona* and *Dendrocalamus* showed maximum amount of residual lead. The residual accumulation values were in decreasing order. *Phyllanthus*, *Commelina*, *Ocimum* and *Vinca* showed interestingly lower lead accumulation and the values were in the increasing order. Maximum amount of lead to the tune of 3.62 ± 0.16 mg of lead/g DW of leaf sample was observed in *Mangifera indica*, L. belonging to the family-Anacardiaceae. *Psidium guajava*, L. (F: Myrtaceae) showed 3.18 ± 0.38 mg of lead/g DW of leaf sample; *Michelia champaca*, L. (F: Magnoliaceae) showed 2.93 ± 0.54 mg of lead/g DW of leaf sample and *Tectona grandis*, L.f. (F: Verbenaceae) showed 2.85 ± 0.27 mg of lead/g DW of leaf sample. It was observed that perennial trees accumulated more amount of lead than shrubs and herbs. Herbs accumulated the least amount of lead, when compared to shrubs and trees. The plants collected from Karanjei forest area, did not show any accumulation of lead and if at all present lead was not traceable. Minimum lead accumulation was observed in case of *Phyllanthus reticulatus*. Minimum amount of lead to the tune of 0.45 ± 0.11 mg of lead/g DW of leaf sample was observed in *Phyllanthus reticulatus*, Poir belonging to the family- Euphorbiaceae. However, the plants collected from the Karenjei area (Ganjam district) did not show any residual lead level in leaf tissues indicating non contamination which served as control for comparison. The pigment content of leaves of the plants sampled

from the toxicant exposed area (roadside) were compared with the pigment content of the plant leaf samples collected from a non contamination area. Variation in pigment contents were recorded in control and contaminated plant leaf samples. In all exposed cases tested, significant decrease in chlorophyll content was recorded when compared to their respective control values. Maximum decrease in chlorophyll content was recorded in case of *Commelina benghalensis* to the tune of 56.3% decrease, where the chlorophyll content depleted from $1.35 \pm 0.15 \text{mg/g FW}$ to $0.59 \pm 0.21 \text{mg/g FW}$, followed by 27.2% decrease in chlorophyll content in *Oldenlandia corymbosa*, where the chlorophyll content depleted from $1.25 \pm 0.33 \text{mg/g FW}$ to $0.91 \pm 0.24 \text{mg/g FW}$. Minimum decrease in chlorophyll content was recorded in case of *Datura stramonium* to the tune of 2.5% where the chlorophyll content depleted from $2.41 \pm 0.19 \text{mg / g FW}$ to $2.35 \pm 0.34 \text{mg/g FW}$, followed by 5.8% decrease in chlorophyll content in *Ervatamia divaricata*, where the chlorophyll content depleted from $1.74 \pm 0.23 \text{mg/g FW}$ to $1.64 \pm 0.18 \text{mg/g FW}$. Similar pattern was observed in case of carotene pigment analysis. In all the plants tested, depletion in carotene value was observed in contaminated leaf samples when compared to exposed leaf samples except in case of *Ocimum sanctum*, *Cascabela thevtia*, and *Tectona grandis* an increase in carotene content was observed which was not notable. In rest of the plants tested decrease in carotene content was marked. Maximum depletion in carotene content was recorded in case of *Morind citrifolia*, followed by *Costus speciosus*, followed by *Datura stromonium* and *Cleome viscose* followed by *Ficus benghalensis* and minimum depletion was recorded in case of *Euphorbia hirta*, where 3.4% depletion was noted.

All the plants showed decrease in pigment content with the increase in residual lead concentration except few where increase in carotene level was marked. The percent decrease of pigment content did not follow any significant trend and the regression value was non significant. However, all the pigment values decreased in the plant leaves collected from the road sides of the national highways when compared to control. The perennial trees showed higher accumulation of lead but this residual lead could not induce significant changes in the pigment content. The leaves of some plants turned brown and black and blackening of leaves was observed and maximum dust was deposited on the leaves. We have also marked leaf fall in addition to regular leaf fall in some plants. At present the situation may not look grim but in future with higher accumulation of lead and other chemicals coming out as exhaust of the vehicles may lead to destruction and disappearance of plants on the roadsides leading to desertification of the roadsides. Now lead free fuel is available in the market, which is generally used by all the cars, buses and trucks. But use of kerosene by the buses and trucks create wide spread air pollution problem. These vehicles discharge huge amount of gases as exhaust. The plants collected from the Karenjei area did not show any residual lead level in leaf tissues indicating non contamination which served as control for comparison. However, the plants collected from the sides of National Highways (NH) contained residual lead in their leaf tissues.

Toxicity testing was carried out for petriplate culture under laboratory controlled conditions. In case of petriplate culture, the MAC value was 1.1mg / l , LC_{10} was 2.53mg / l , LC_{50} was 7.54mg / l , LC_{90} was 17.8mg / l and LC_{100} was 25.2mg / l when we consider percentage of seed germination. However, when we considered seedling establishment, the values significantly changed. The maximum allowable concentration (MAC) for ragi seeds in petriplate culture, calculated from seedling establishment was found to be 0.51mg / l , LC_{10} was 1.73mg / l , LC_{50} was 5.2mg / l , LC_{90} was 13.75mg / l & LC_{100} was 15.0mg / l , when we consider percentage of seedling establishment in petriplates as a single dose. Experiments were conducted at 14.6mg of lead nitrate kg^{-1} dry soil mixture, as MAC dose.

The total chlorophyll content increased from $0.246 \pm 0.091 \text{mg}$ of chlorophyll / g FW to $0.311 \pm 0.072 \text{mg}$ of chlorophyll / g FW at 0.51mg of lead / liter (maximum allowable concentration- MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Table- 1). The total chlorophyll content increased from $0.245 \pm 0.032 \text{mg}$ of chlorophyll / g FW to $0.249 \pm 0.019 \text{mg}$ of chlorophyll / g FW at 1.73mg of lead / liter (lethal concentration with 10% death- LC_{10}) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Table- 1). The total chlorophyll content decreased from $0.246 \pm 0.041 \text{mg}$ of chlorophyll / g FW to $0.168 \pm 0.036 \text{mg}$ of chlorophyll / g FW at 5.20mg of lead / liter (lethal concentration with 50% death- LC_{50}) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. At MAC value and LC_{10} value increase in chlorophyll content was recorded and at LC_{50} decrease in chlorophyll content in lead nitrate exposed seedlings was noted. With the increase in lead nitrate doses the total chlorophyll content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions (Table- 1). At MAC value and LC_{10} value increase in chlorophyll content was recorded and at LC_{50} decrease in chlorophyll content in lead nitrate exposed seedlings was noted.

Table-1: Showing changes in pigment content in control and lead nitrate exposed 144h seedlings at different lethal concentration values. Data indicate mean of the samples \pm standard deviation. Values in parentheses indicate percent change in exposed values compared to control values.

| Pigment | Status | Toxicity value of lead nitrate on ragi seedlings |
|---------|--------|--------------------------------------------------|
|---------|--------|--------------------------------------------------|

| content | | MAC | LC ₁₀ | LC ₅₀ |
|----------------------------|---------|---------------------|---------------------|-----------------------|
| Chlorophyll, mg / g FW. | Control | 0.246 ± 0.091 | 0.245 ± 0.032 | 0.246 ± 0.041 |
| | Exposed | 0.311±0.072 (+26.4) | 0.249± 0.019 (+1.6) | 0.168 ± 0.036 (-31.7) |
| Phaeophytin, mg / g FW | Control | 0.212 ± 0.054 | 0.213 ± 0.025 | 0.211 ± 0.028 |
| | Exposed | 0.224± 0.036 (+5.7) | 0.229± 0.028 (+7.5) | 0.232 ± 0.045 (+9.5) |
| Carotenoid mg / g FW. | Control | 0.003 ± 0.0011 | 0.003 ± 0.0016 | 0.003 ± 0.0018 |
| | Exposed | 0.003 ± 0.0016 (0) | 0.003 ± 0.0014 (0) | 0.003 ± 0.0009 (0) |

The total phaeophytin content increased from 0.212±0.054mg of phaeophytin / g FW to 0.224± 0.036mg of phaeophytin/gFW at 0.51mg of lead / liter (maximum allowable concentration- MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The total phaeophytin content increased from 0.213 ± 0.025mg of phaeophytin/g FW to 0.229±0.028mg of phaeophytin / g FW at 1.73mg of lead / liter (lethal concentration with 10% death- LC₁₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Table- 1). The total phaeophytin content increased from 0.211±0.028mg of phaeophytin / g FW to 0.232±0.045mg of phaeophytin / g FW at 5.20mg of lead / liter (lethal concentration with 50% death- LC₅₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. At MAC value, LC₁₀ and LC₅₀ value increase in phaeophytin content was recorded in lead nitrate exposed seedlings. With the increase in lead nitrate doses the total phaeophytin content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. The ANOVA analysis indicated the existence of significant differences between rows and columns. The variation observed is statistically significant.

The biomolecular analyses indicated very interesting results. Change in the DNA content from 0.35±0.09mg/gFW to 0.35±0.12mg/gFW at 0.51mg of lead / liter (maximum allowable concentration- MAC) of lead nitrate was noted in the leaves of 144h old ragi seedlings in petriplate culture (Fig. 1). The DNA content decreased from 0.35±0.09mg/gFW to 0.31± 0.07mg/g FW at 1.73mg of lead / liter (10% lethal concentration, LC₁₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The DNA content significantly decreased from 0.35±0.09mg/gFW to 0.16±0.04mg/gFW at 5.2mg of lead / liter (50% lethal concentration, LC₅₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Fig. D4). The DNA content significantly decreased from 0.35±0.09mg/gFW to 0.08±0.03mg/g FW at 13.75mg of lead / liter (90% lethal concentration, LC₉₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The DNA content significantly decreased from 0.35 ± 0.09mg / g FW to 00mg/gFW at 15.0mg of lead / liter (100% lethal concentration, LC₁₀₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture showing the death of the seedlings. At MAC value very insignificant increase in DNA content was recorded and after LC₁₀ value onwards decrease in DNA content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the DNA content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions (Fig. 1). The regression analysis indicated existence of significant correlation ($p \geq 0.05$). The ANOVA test indicated the existence of significant difference between rows and columns. The RNA content increased from 18.4±3.6mg/gFW to 24.8±4.7mg/gFW at 0.51mg of lead / liter (maximum allowable concentration- MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture, where no change in mean value was noted (Fig. 2). The RNA content decreased from 18.4±3.6mg/g FW to 19.1±2.8mg/g FW at 1.73mg of lead / liter (10% lethal concentration, LC₁₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The RNA content significantly decreased from 18.4±3.6mg/gFW to 11.5±4.9mg/gFW at 5.2mg of lead / liter (50% lethal concentration, LC₅₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The RNA content significantly decreased from 18.4±3.6mg/ gFW to 2.2± 0.8mg/gFW at 13.75mg of lead / liter (90% lethal concentration, LC₉₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The RNA content significantly decreased from 18.4±3.6mg/gFW to 00mg/gFW at 15.0mg of lead / liter (100% lethal concentration, LC₁₀₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture showing the death of the seedlings. At MAC value highly significant increase in RNA content was recorded and after LC₁₀ value onwards notable decrease in RNA content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the RNA content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. The regression analysis indicated existence of non significant correlation ($p \geq NS$). The ANOVA test indicated the existence of significant difference between rows and columns.

Fig.1: Changes in DNA content in control and lead nitrate exposed 144hr seedlings in petriplate culture.

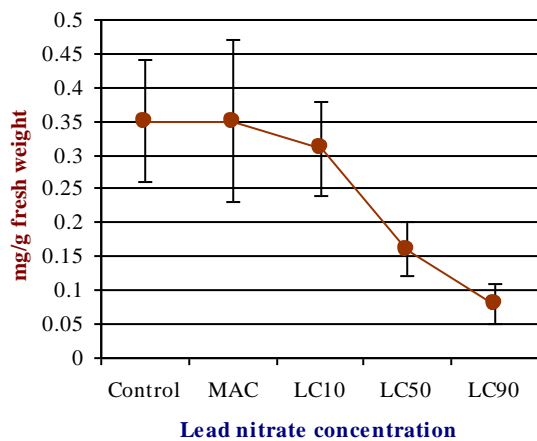


Fig. 2: Changes in RNA content in control and lead nitrate exposed 144hr old seedlings in petriplate culture.

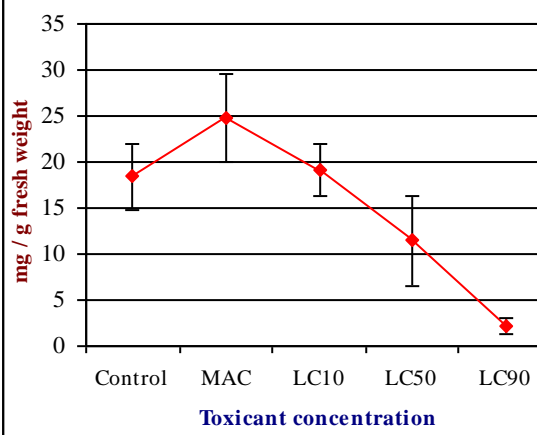


Fig. 3: Changes in Protein content of control and lead nitrate exposed 144hr old seedlings in petriplate culture.

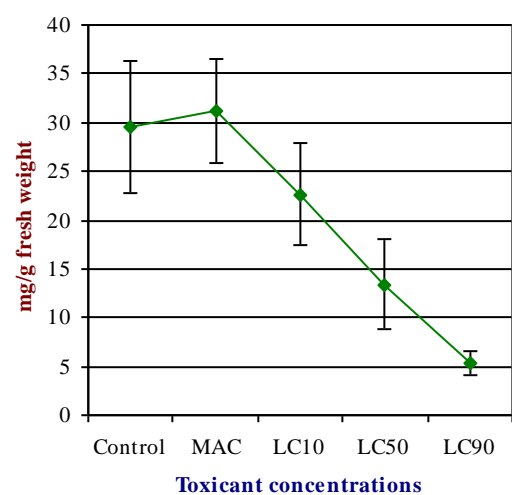
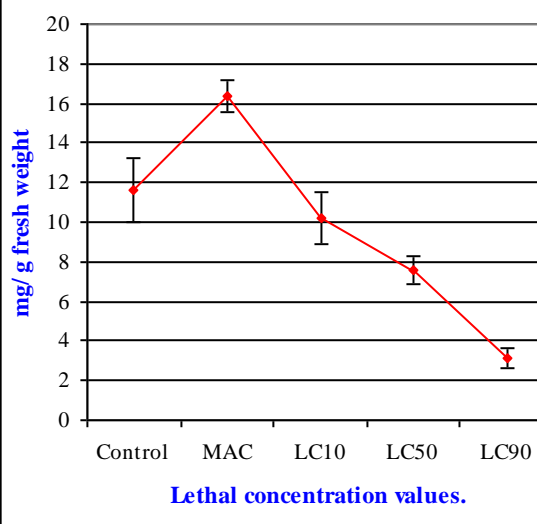


Fig.4: Changes in FAA content in control and lead nitrate exposed 144hr old seedlings in petriplate culture.



The protein content increased from 29.6 ± 6.8 mg/g FW to 31.2 ± 5.4 mg/g FW at 0.51 mg of lead / liter (maximum allowable concentration- MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture, where no change in mean value was noted (Fig. 3). The protein content decreased from 29.6 ± 6.8 mg/g FW to 22.6 ± 5.2 mg/g FW at 1.73 mg of lead / liter (10% lethal concentration, LC₁₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The protein content significantly decreased from 29.6 ± 6.8 mg/gFW to 13.4 ± 4.6 mg/g FW at 5.2 mg of lead / liter (50% lethal concentration, LC₅₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Fig. D6). The protein content significantly decreased from 29.6 ± 6.8 mg/gFW to 5.4 ± 1.2 mg/gFW at 13.75 mg of lead / liter (90% lethal concentration, LC₉₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The protein content significantly decreased from 29.6 ± 6.8 mg/gFW to 00 mg/gFW at 15.0 mg of lead / liter (100% lethal concentration, LC₁₀₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture showing the death of the seedlings (Fig. 3). At MAC value highly significant increase in protein content was recorded and after LC₁₀ value onwards notable decrease in protein content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the protein content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. The regression analysis indicated existence of insignificant correlation ($p \geq 0.05$). The ANOVA test indicated the existence of significant difference between rows and columns. The FAA content increased from 11.6 ± 1.2 mg/g FW to 16.4 ± 1.8 mg/gFW at 0.51 mg of lead / liter (maximum allowable concentration- MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture, where no change in mean value was noted (Fig. 4). The FAA content decreased from 11.6 ± 1.2 mg/gFW to 10.2 ± 2.2 mg/gFW at 1.73 mg of lead / liter (10% lethal concentration, LC₁₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The FAA content significantly decreased from 11.6 ± 1.2 mg/gFW to 7.6 ± 1.1 mg/gFW at 5.2 mg of lead / liter (50% lethal concentration, LC₅₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The FAA content significantly decreased from 11.6 ± 1.2 mg/gFW to 3.1 ± 0.9 mg/gFW at 13.75 mg of lead / liter (90%

lethal concentration, LC₉₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The FAA content significantly decreased from 11.6±1.2mg/gFW to 00mg / g FW at 15.0mg of lead / liter (100% lethal concentration, LC₁₀₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture showing the death of the seedlings. At MAC value highly significant increase in FAA content was recorded and after LC₁₀ value onwards notable decrease in FAA content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the FAA content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions (Fig. 4). The regression analysis indicated existence of insignificant correlation ($p \geq 0.05$). The ANOVA test indicated the existence of significant difference between rows and columns.

Table-2: Showing residual lead in root, shoot and whole lead nitrate exposed 144h old seedlings in petriplate culture. NT= Not traceable. Data are the mean of the samples \pm standard deviation. MAC- Maximum allowable concentration, LC-lethal concentration.

| Lethal concentration values | Lead nitrate concentrations mg of lead / liter | Residual lead mg / g Fresh Weight | | |
|-----------------------------|------------------------------------------------|-----------------------------------|-------------|----------------|
| | | Root | Shoot | Whole seedling |
| Control | 0.00 | NT | NT | NT |
| MAC | 0.51 | 0.045±0.011 | 0.036±0.009 | 0.083±0.017 |
| LC ₁₀ | 1.73 | 0.054±0.014 | 0.043±0.019 | 0.096±0.024 |
| LC ₅₀ | 5.20 | 0.066±0.009 | 0.058±0.014 | 0.112±0.016 |
| LC ₉₀ | 13.75 | 0.069±0.011 | 0.072±0.018 | 0.132±0.021 |
| LC ₁₀₀ | 15.00 | 00 (NT) | 00(NT) | 00(NT) |

Table-2 shows residual lead in root, shoot and whole lead nitrate exposed 144h old seedlings in petriplate culture. No residual lead was detected in the root, shoot and whole seedling of the control set. In case of exposed roots, significant amount of lead has accumulated after absorption from the petriplates. At sub-lethal concentration (MAC) 0.045±0.011mg of lead / g FW was recorded. At LC₁₀, lead to tune of 0.054±0.014 mg of lead / g FW accumulated, at LC₅₀, lead to tune of 0.066±0.009mg of lead / g FW accumulated, at LC₉₀ lead accumulated to the tune of 0.069±0.011mg of lead / g FW and at LC₁₀₀, as there was no germination and seedling establishment, hence there was no accumulation of lead in the roots. In shoot of the control seedlings, no residual mercury was detected. In case of exposed shoots, significant amount of lead has accumulated after absorption by root and translocated to shoot from the petriplates. At sub-lethal concentration (MAC) 0.036±0.009mg of lead / g FW was recorded. At LC₁₀, lead to tune of 0.043±0.019mg of lead / g FW accumulated, at LC₅₀, lead to tune of 0.058±0.014mg of lead / g FW accumulated, at LC₉₀ lead accumulated to the tune of 0.072±0.018mg of lead / g FW and at LC₁₀₀, as there was no germination and seedling establishment, hence there was no accumulation of lead in the shoots. In whole control seedlings, no residual mercury was detected. In case of exposed whole seedling, significant amount of lead has accumulated after absorption from the petriplates. At sub-lethal concentration (MAC) 0.083±0.017mg of lead / g FW was recorded. At LC₁₀, lead to tune of 0.096±0.024mg of lead / g FW accumulated, at LC₅₀, lead to tune of 0.112±0.016mg of lead / g FW accumulated, at LC₉₀ lead accumulated to the tune of 0.132±0.021mg of lead / g FW and at LC₁₀₀, as there was no germination and seedling establishment, hence there was no accumulation of lead in the whole seedling. (Table-2). From the observations and results, it is clear that lead nitrate is deadly toxic and can significantly affect any plant or animal.

Discussion

The present piece of work has an applied value as lead is freely available as a regular air contaminant generally discharged from automobiles as exhaust into air, in addition to many other sources of lead discharge in to the environment. Lead is known as a serious pollutant and causes serious hazards to all organisms (Sahu & Panigrahi, 2015). The old plant leaves collected from the roadsides of freeways and national highways showed significant amount of lead. We could not record presence of lead in younger plant leaves and young plants either planted or grown naturally. Non availability of lead in the young plant leaves was probably due to non use lead in petroleum products like diesel and petrol in vehicles. These young plants suffer from vehicular exhausts where yellowing of leaves and stunted growth was noticed but residual lead accumulation was not marked. Minimum lead accumulation was observed in case of *Phyllanthus reticulatus*. These young plants were planted only in the last 10years and in the last 10years lead was phased out and unleaded petrol and diesel was sold in the market for use as fuel in transporting vehicles. The automobile exhausts no more contain lead. Most of the older plants left out during road expansion contained lead. It was clear that at present the situation of both the ghat sections is not grim. One

important point note worthy is that the old leaves of the plants containing residual lead when falls in summer, these old leaves decay on soil and accumulated lead then becomes free and available on the soil. These lead compounds can leach to the bottom of the soil enriching the field again with lead or the free lead compounds can travel to distances with rain run off water and can enrich crop fields. The availability of lead by decay and decomposition, followed by transport to different areas with rain run off water raises concern. Significant depletions in the pigment content of both contaminated plants of the roadside and the exposed seedlings of the petriplate culture indicated the impact of lead present in the air and the lead applied on the petriplates. The percent decrease of pigment content did not follow any significant trend and the regression value was non significant. However, all the pigment values decreased in the plant leaves collected from the road sides of the national highways. The leaves of some plant looks brown and black and we have observed blackening of leaves and dusts were deposited on the leaves. We have also marked leaf fall in addition to regular leaf fall in some plants. Let us not presume that the non significant decrease in parameters is a good sign but the observed variations indicate stress and at higher exposure period the plants may show signs of toxicity. At present it may not look grim but in future with higher accumulation of lead may lead to destruction and disappearance of plants on the roadsides. Kopittke *et al.*, (2008) reported that unpropitious effects on germination and growth can be marked in lead exposed plants at micromolar level. We respectfully beg to differ from this information as we have found a different result. In the present study, it was observed that at very low sub-lethal concentration of lead nitrate, enhancement in root and shoot growth was marked initially but at higher exposure period decrease in parameters was marked. Beyond sub-lethal concentration, lead nitrate severely affected the germination and seedling growth of ragi seeds. Similar results were also observed in case of alumina poisoning in case of green gram and mercury contained effluent poisoning in case of rice. Islam *et al.* (2008) reported well defined inhibition in germination at very low doses of Lead²⁺. Inhibition and depletion of seed germination, growth root and aerial part shoot was reported in plants like wheat, rice, maize etc. induced by lead (Islam *et al.*, 2008). The same author also indicated that at higher concentrations of lead acceleration of germination can be marked and simultaneously adverse affects on the radicle and hypocotyl length of *E. argy* can be seen. Lead pollution may also show the lead exposed roots to swell, bend, short and stubby and also can show higher number of secondary roots (Kopittke *et al.*, 2008). Higher doses of lead can significantly reduce plant biomass (Singh *et al.*, 2010). Arias *et al.* (2010) reported inhibition of root elongation significantly in Mesquite (*Prosopis* sp.). At MAC value very insignificant increase in DNA content was recorded and after LC₁₀ value onwards decrease in DNA content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the DNA content decreased in 144h old ragi seedlings in petriplate culture. At MAC value highly significant increase in RNA content was recorded and after LC₁₀ value onwards notable decrease in RNA content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the RNA content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. At MAC value highly significant increase in protein content was recorded and after LC₁₀ value onwards notable decrease in protein content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the protein content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. At MAC value highly significant increase in FAA content was recorded and after LC₁₀ value onwards notable decrease in FAA content was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the FAA content decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. Highly significant decreased in DNA, RNA, protein and FAA content was observed at LC₅₀, LC₉₀ and LC₁₀₀ of lead nitrate in petriplate culture under laboratory controlled conditions. At LC₉₀ of lead nitrate, the DNA, RNA, protein and FAA content decreased by 77.1%, 88.1%, 81.8% and 73.3% decrease over control value was marked. From the data it is evident that lead nitrate interferes in biochemical synthesis of nucleic acids. The initial increase in all the four biomolecules tested except DNA might be due to some short of stimulation in biochemical metabolism. Lead is not a known stimulator but by some short of triggering (yet to be found out / not known) in metabolic processes increase in biomolecular content was recorded. This aspect needs detailed further study for confirmation.

It was observed that the root of the exposed seedlings accumulated higher amount of lead when compared to shoot / leaf. Lane and Martin (1977) conducted lead uptake studies and opined that roots have the ability to absorb significant amount of lead and also restrict translocation of lead to aerial parts or above ground parts. In the present study, it was marked that roots absorbed lead from the soil direct and the same metal was translocated to shoot and leaf, as there was no aerial absorption of lead from the environment. Restriction of lead translocation was not marked in the present study. The data was very clear and we found residual lead both in the root and the shoot or seedling leaves in the early part of germination in petriplate culture studies. In case of pot culture studies, we have also observed residual lead in root, shoot and leaf in early and late parts of seedling growth. Hence it can be inferred that the total lead was only absorbed from

the inoculated medium and there was no aerial absorption of lead. Under laboratory control conditions and at normal room temperature, pressure and humidity lead will not evaporate from the inoculated medium and also there were no other biological agents who vaporize the metal lead or its compounds. This experiment was conducted under laboratory controlled conditions and aerial availability of lead was restricted. The belief that root will restrict translocation of lead to aerial part was over turned by Miller and Koepp (1971) and our data observed recently also agree with Miller and Koepp (1971). Aerial deposition of lead and subsequent entry of lead through the pores into the leaf can not be ruled out. But the quantity of lead entry through the pore is a question to be answered in future. However, Godzik, (1993) clearly hinted to the extent to which lead can enter in to plant body via the leaves depends on the ability of leaves to absorb lead or lead to penetrate into leaf from aerial sources in case of Downy leaves which depends on the specific leaf morphology, number of stomata present and most important environmental conditions. Kumar *et al.*, (1995) agreed that the major portion of the lead absorbed by plants remains in the roots only. In our experiment, we have observed higher accumulation of lead in roots only in growing plants. We have not tested the retention time of lead in the root and the reasons of non translocation of this metal to the aerial parts of the plant. In growing seedlings and plants, it was not possible to assay and understand the mechanism of retention and translocation of this metal. Interestingly, Ghani (2010) also indicated that in all lead treatments, in case of Desi, the accumulated lead level in roots was higher when compared to the roots of Neelam variety. The same author probably agreed that Desi can translocated lead better than Neelam but in both the varieties lead can be translocated from root to the aerial part after absorption by root. Fritioff and Greger (2006) reported the immobility of lead in plants, which we are inclined not to accept. Our results in the present study indicated the presence of lead and accumulation of lead in the leaves of the ragi seedlings both in petriplate culture and pot culture after absorption by the roots from inoculated medium in petriplates or from the inoculated soil of pot culture. Ghani (2010) also opined that the limited /restricted movement of lead from roots to the leaves was probably due to the barrier of the root endodermis, which we agree partly but this is yet to be confirmed in our experiment. The root was more affected than the shoot. Fahr *et al.*, (2013) reported that “the primary effect of lead toxicity in plants is a rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip” (Eun *et al.*, 2000). Samardakiewicz and Wozny, (2005) practically exhibited that lead caused inhibition of cell division in *Lemna minor* roots. The similar type was demonstrated in several plant species, including *Triticum aestivum* by Kaur *et al.*, (2013). Reduction in root length and dry weight influenced by lead intoxication was reported by Munzuroglu and Geckil (2002). Fahr *et al.*, (2013) clearly explained the mechanism of lead absorption by the roots from soil by taking the information given by few workers along with their views and findings listed below. Lynch and Whipps (1990) indicated that the rhizosphere was the place exchange and interaction might have taken place. Blaylock and Huang (2000) predicted that lead had low solubility and less availability for uptake of the plant because it produces by chemical interaction and precipitates with inorganic chemicals forming complexes and binds with organic chemicals in the rhizosphere zone. The excretory exudates and several metabolites were excreted from root could react with environmentally available chemical at root-soil zone and where the soil pH is changed. This will not permit the formation of soluble metal-organic complex, a process which will not allow absorption of the heavy metal (Fahr *et al.*, 2013 & Leyval and Berthelin, 1991). Mench *et al.*, (1987) reported that “citric, fumaric, and uronic acids as well as many polysaccharides are able to form complexes and to chelate metal ions including lead”. Fahr *et al.*, (2013) indeed informed that “in *Vicia faba* and *Typha angustifolia*, lead uptake by roots was shown to increase significantly in the first hour after adding organic ligands [ethylenediaminetetraacetic acid (EDTA), citric acid; and this view was well supported by Muhammad *et al.*, (2009) and Shahid *et al.*, (2012)”. At LC₀₀ (MAC) lead nitrate dose value, the DNA, RNA, protein and FAA content increased when compared to its respective control values. No change in DNA content was noticed between control and lead nitrate exposed seedlings, at MAC value except a small variation within standard deviation level. When the lead nitrate dose increased to LC₁₀ level, significant decrease in DNA, protein and FAA content was recorded and RNA content increased when compared to control but the RNA content was far less than the MAC value data. Highly significant decreased in DNA, RNA, protein and FAA content was observed at LC₅₀, LC₉₀ and LC₁₀₀ of lead nitrate in petriplate culture under laboratory controlled conditions. At LC₉₀ of lead nitrate, the DNA, RNA, protein and FAA content decreased by 77.1%, 88.1%, 81.8% and 73.3% decrease over control value was marked. From the data it is evident that lead nitrate interferes in biochemical synthesis of nucleic acids. The initial increase in all the four biomolecules tested except DNA might be due to some short of stimulation in biochemical metabolism. Lead is not a known stimulator but by some short of triggering (yet to be found out / not known) in metabolic processes increase in biomolecular content was recorded. This aspect needs detailed further study for confirmation. Higher doses of lead nitrate and higher exposure period drastically influenced the photosynthetic rate, respiration rate and gross primary production of the ragi plant

compared to control ragi plant. Initial exposure of ragi seedlings to lead nitrate indicated no change in DNA content but the DNA content of 30th day old seedlings of pot culture decreased in pot culture indicating either cell death or non biosynthesis of DNA. 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. But at higher exposure period and higher doses of toxicant depletion in RNA content was noticed. 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. 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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Declarations

Author contribution statement

Prof. A.K. Panigrahi: Conceptualization, planning and execution of the project, Original draft preparation, supervision, reviewing and editing; Dr. Alaka Sahu- Planning and execution of the project, Manuscript preparation, supervision, reviewing and editing Research work conducted by scholar, Sabita Barik-collection of environmental samples, analysis and related field work. laboratory experimental work, preparation of draft and editing Ms. Barik contributed reagents & glassware for laboratory experiment related work and field study expenses.

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