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Eco-toxicological impact of atmospheric lead on road side perennial plants and ragi seedlings in petri plate culture under controlled conditions

Sabita Barik*, Alaka Sahu and A. K. Panigrahi

Environmental Toxicology & Biotechnology Laboratory, Department of Botany, Berhampur University, BERHAMPUR 760 007, Odisha, India *K.S.U.B. College, Bhanjanagar 761 126, Ganjam, Odisha, India

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ABSTRACT

Higher accumulation of lead in perennial old plant leaves were observed. Young plants either showed no residual lead or the amount of lead if present was insignificant. No residual lead was observed in small herbs present on both the sides of the road. Significant decrease in total chlorophyll and phaeophytin content was recorded in plant leaves situated on the roadsides of the ghat area when compared to plants collected from deep inside the forest area far away from freeways / highways. Insignificant decrease or no change in carotenoid content was recorded in plant leaves collected from the plants available on the roadsides of the ghat area. Change in leaf colour and heavy surface deposition was noted in exposed leaves of the plants. Residual lead level was untraceable in control plant leaves. Initial increment in photosynthetic rate was observed at LC_{00} (MAC) of lead nitrate in petriplate culture compared to control value followed by depletion of photosynthetic rate in higher lead nitrate concentrations in petriplate culture. Tissue respiration and photosynthesis rate significantly declined in lead exposed seedlings in petriplate culture experiments depleting the productivity of the exposed seedlings when compared to control seedlings. The GPP value decreased in all exposed seedlings indicating the impact of the toxicant. Notable but insignificant amount of residual lead was noted in lead nitrate exposed 6day old seedlings in petriplate culture. The observed observation in all parameters studied relates to residual lead accumulation in the seedlings which might have impacted different physiological activity in the exposed ragi seedlings.

Key words: Pollution, Residual lead, Ragi, Photosynthetic rate, Respiration rate

Introduction

Pollution is considered to be a creation of man or man's actions and related to direct or indirect human activity. Most of the air pollution is caused by the automobiles in addition to industrial exhausts. The intensity of air pollution is very high in and around National highways and freeways and in city traffics. The intensity is dependent on the traffic flow and the type of vehicles. Trucks, buses, trekkers, dumpers etc discharge huge amount of exhausts and mostly in these vehicles kerosene is used as fuel instead of diesel. The automobiles discharge a huge amount of exhausts containing poisonous gases including lead and some other chemicals (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 contami-

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nated 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). The same authors also reported that the gaseous chemicals coming out as automobile exhausts affect land plants and the pigment system was badly affected. The impact was probably due to lead accumulation in plants as reported by the same authors. Literature pertaining to impact of lead and its compounds on crop plants is scanty. The present piece of work has been planned to find out the residual lead present on the old roadside plants and its effect on pigment content of the plants collected from NH road sides in a ghat section at Kalinga (NH-157) and Keshpur at NH-16. An attempt was made to study the impact lead on the metabolic activity of the ragi seedlings in petriplate culture under laboratory controlled conditions.

Study site: Karenjei deep forest far away from the roadways was selected as control site for comparision (Photo-1).Two ghat sections located on two different National Highways. NH-157 (Photo-2), NH-16 (Photo-3) and Narayani temple site (Photo-4) little away from NH-16 but heavily contaminated site by vehicles were selected for the study sites. Leaf samples were collected for residual lead analysis and pigment estimations to observe the impact lead along with other air pollutants released from vehicles.

Materials and Methods

The leaf samples collected from the contaminated site and control and lead nitrate exposed 144hr old ragi seedling leaves were analyzed for pigment following the method described by Vernon (1960) and



Photo-1. 2. Durgaprasad 3. Keshpur 4. Narayani Karenjei site ghat ghat site



(Photo-5: Thinning of road side forest

6. Deposits on the leaf, browning of leaf)

Davies (1976). The obtained data were statistically analyzed. The leaf samples collected from the contaminated field and ragi seedlings were digested in a Klein's apparatus with acid digestion mixture, cooled and residual lead level was measured in an Atomic Absorption Spectrophotometer (Yoshida et al., 1976) and expressed as mg of lead g⁻¹ dry weight of the samples (Wantorp and Dyfverman (1955) as modified by Barik and Sahu (2017). The evolution of carbon dioxide and evolution of O₂ in Photosynthesis were estimated manometrically with the help of a Photo-warburg's apparatus adopting the technique of Hannan and Patouillet (1972) and Oser (1965). The obtained manometric values were calculated taking Flask's constant and photosynthetic rate and respiration rate was computed and shown as ml of O₂ evolved / hr / g and in ml of CO₂ evolved / h / g of leaf of the seedlings, respectively. The obtained data was statistically analyzed.

Results

Thinning of forest was observed on both the air polluted sites of Keshpur ghat area and Durgaprasad ghat area (Photo-5), compared to Karenjei forest area where thick forest was observed. In the contaminated site, it was observed that perennial plant leaves were heavily coated with dust and pollutants (Photo-6). Browning of leaves was observed in the contaminated site. In normal forest when a plant

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leaf matures, yellowing of the leaf followed by leaf fall was noted. But in contaminated site heavily coated young leaves were found on the ground. Leaf samples of the control site and contaminated sites were collected for pigment analysis to understand the impact lead contained air pollutants on the plant leaves.

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 ± 0.15 mg / gFW) to 0.59 ± 0.21 mg / gFW, followed by 27.2% decrease in chlorophyll content in Oldenlandia corymbosa, where the chlorophyll content depleted from 1.25 ± 0.33 mg / gFW to 0.91 ± 0.24 mg / gFW. 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 ± 0.19 mg / g FW to 2.35 ± 0.34 mg / g FW, followed by 5.8% decrease in chlorophyll content in Ervatamia divarcata, where the chlorophyll content depleted from 1.74 ± 0.23 mg / gFW to 1.64 ± 0.18 mg / gFW. In all exposed cases significant decrease in phaeophytin content was recorded when compared to their respective control value, except in Catharanthus roseus where an insignificant increase in phaeophytin to the tune of 1.6% was noted (Fig.1).



Fig. 1. Relationship of residual lead in contaminated plant leaves with percent changes of leaf pigments.

Maximum decrease in phaeophytin content was recorded in case of *Commelina benghalensis* to the tune of 52.1% decrease, where the phaeophytin content depleted from 0.96 ± 0.28 mg g⁻¹FW to $0.46 \pm$

0.08 mg / g FW, followed by 29.8% decrease in phaeophytin content in Phyllanthus reticulatus, where the phaeophytin content depleted from 0.94±0.08 mg / g FW to 0.66±0.11 mg / g FW. Minimum decrease in phaeophytin content was recorded in case of Mangifera indica to the tune of 1.7% decrease, where the phaeophytin content depleted from 0.936 \pm 0.11 mg g⁻¹ FW to 0.92 \pm 0.18 mg / g FW, followed by 1.8% decrease in phaeophytin content in Datura stromonium, where the phaeophytin content depleted from 1.65 ± 0.27 mg / g FW to 1.62±0.38 mg g⁻¹FW (Table 1). Interestingly, in case of Tectona grandis, the phaeophytin content showed an insignificant depletion (3.4%) when compared to control leaf sample. 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.

Experimental studies

Toxicity testing was carried out for petriplate culture under laboratory controlled conditions. In case of petriplate culture, the MAC value was 1.1 mg / l, LC_{10} was 2.53 mg / l, LC_{50} was 7.54 mg/l, LC_{90} was 17.8 mg / l and LC₁₀₀ was 25.2 mg / 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 0.51 mg / l, LC_{10} was 1.73 mg / l, LC $_{50}$ was 5.2 mg / l, LC $_{90}$ was 13.75 mg / l and LC_{100} was 15.0 mg / 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. Fig. D1 showed 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±0.011 mg of lead / g FW was recorded. At LC_{10} lead to tune of 0.054±0.014 mg of lead / g FW accumulated, at LC_{50} lead to tune of 0.066±0.009 mg 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±0.009 mg of lead / g FW was recorded. At LC_{10} lead to tune of 0.043±0.019 mg of lead / g FW accumulated, at LC₅₀ lead to tune of 0.058 ± 0.014 mg of lead / g FW accumulated, at LC₉₀ lead accumulated to the tune of 0.072±0.018 mg of lead / g FW and at LC_{100} 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±0.017 mg of lead / g FW was recorded. At LC_{10} lead to tune of 0.096±0.024 mg of lead / g FW accumulated, at LC_{50} lead to tune of 0.112±0.016mg of lead / g FW accumulated, at LC₉₀ lead accumulated to the tune of 0.132±0.021 mg 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 (Fig. D1).



The photosynthetic rate increased from $168.5 \pm 18.4\mu$ l of oxygen evolved / g / hr to $181.4 \pm 21.4\mu$ l of oxygen evolved / g / hr at 0.51mg of lead / liter (MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Fig. D2). The photosynthetic rate decreased from $168.5 \pm 18.4\mu$ l of oxygen evolved / g / hr to $151.2 \pm 24.6\mu$ l of oxygen evolved / g / hr at 1.73 mg of lead / liter (LC₁₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The photosynthetic rate signifi-

cantly decreased from $168.5 \pm 18.4 \,\mu$ l of oxygen evolved / g / hr to $88.4 \pm 16.2 \mu$ l of oxygen evolved / g / hr at 5.2 mg of lead /l (LC₅₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The photosynthetic rate significantly decreased from 168.5 \pm 18.4 µl of oxygen evolved / g / hr to $76.5 \pm 18.2 \,\mu$ l of oxygen evolved/g/hr at 13.75 mg of lead / $l(LC_{00})$ of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The photosynthetic rate significantly decreased from $168.5 \pm 18.4 \,\mu$ l of oxygen evolved/g/hr to 0.0 μ l of oxygen evolved/g/hr at 15.0 mg of lead /l (LC₁₀₀) of lead nitrate in the leaves of 144 h old ragi seedlings in petriplate culture showing the death of the seedlings. At MAC value increase in photosynthetic rate was recorded and at LC₁₀ onwards decrease in photosynthetic rate was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the photosynthetic rate decreased in 144 h old ragi seedlings in petriplate culture under laboratory controlled conditions (Fig. D2).



The respiration rate decreased from 149.5 ± 11.2 μ l of carbon dioxide evolved / g / hr to 148.2 ± 14.8µl of carbon dioxide evolved / g / hr at 0.51mg of lead / 1 (MAC) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture (Fig. D2). The respiration rate decreased from $149.5 \pm 11.2 \ \mu l$ of carbon dioxide evolved / g / hr to $136.4 \pm 18.3 \,\mu$ l of carbon dioxide evolved/g / hr at 1.73 mg of lead/l (LC_{10}) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The respiration rate significantly decreased from 149.5 \pm 11.2 µl of carbon dioxide evolved/g/hr to $68.5 \pm 12.2 \mu$ l of carbon dioxide evolved / g / hr at 5.2mg of lead / liter (LC_{50}) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The respiration rate significantly decreased from 149.5 \pm 11.2 µl of carbon dioxide evolved / g / hr to $49.2 \pm 9.4 \mu$ l of carbon dioxide evolved / g / hr at 13.75 mg of lead /

liter (LC₉₀) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The respiration rate significantly decreased from 149.5 ± 11.2 µl of carbon dioxide evolved / g / hr to 0.0 µl of carbon dioxide evolved / g / hr at 15.0 mg of lead / l (LC₁₀₀) of lead nitrate in 144 h old ragi seedlings in petriplate culture showing the death of the seedlings. At MAC value instead of any increase only decrease in respiration rate was recorded and at LC₁₀ dose onwards decrease in respiration rate was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the respiration rate decreased in 144 h old ragi seedlings in petriplate culture under laboratory controlled conditions (Fig. D2). The regression analysis indicated notable nega-

tive correlation (r = -0.993; P \ge 0.01). The gross primary production rate increased from 318.0µl of oxygen evolved/g/hr to 329.6 µl of oxygen evolved/g/hr at 0.51 mg of lead/l (maximum allowable concentration-MAC) of lead nitrate in the leaves of 144 h old ragi seedlings in petriplate culture (Fig. D3). The gross primary production rate decreased from 318.0 μ l of oxygen evolved/g/h to 287.6 µl of oxygen evolved / g / h at 1.73 mg of lead /l (10% lethal concentration, LC_{10}) of lead nitrate in the leaves of 144 h old ragi seedlings in petriplate culture. The gross primary production rate significantly decreased from 318.0 µl of oxygen evolved/ g/hr to 156.9 µl of oxygen evolved / g / h at 5.2 mg of lead/l (50% lethal concentration, LC_{50}) of lead nitrate in the leaves of 144 h old ragi seedlings in petriplate culture. The gross primary production rate significantly decreased from 318.0 µl of oxygen evolved/g/h to 125.7 μl of oxygen evolved / g / h at 13.75 mg of lead/l (90% lethal concentration, LC_{00}) of lead nitrate in the leaves of 144h old ragi seedlings in petriplate culture. The gross primary production rate significantly decreased from 318.0 µl of oxygen evolved/g/h to 0.0 µl of oxygen evolved / g / h at 15.0 mg of lead/l (100% lethal concentration, LC_{100}) of lead nitrate in the leaves of 144 h old ragi seedlings in petriplate culture showing the death of the seedlings (Fig. D3). At MAC value insignificant increase in gross primary production rate was recorded and after LC₁₀ value onwards decrease in gross primary production rate was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the gross primary production rate decreased in 144 h old ragi seedlings in petriplate culture under laboratory controlled conditions (Fig. D3). The photosynthetic rate



and gross primary production rate values increased at MAC value of lead nitrate in 144 h old ragi seedlings compared to their respective control values but increase was not statistically significant. The photosynthetic rate of 144 h old ragi seedlings depleted by 10.3%, 47.5%, 54.6% and 100% inn LC_{10} , LC_{50} , LC_{90} and LC₁₀₀ doses of lead nitrate in petriplate culture respectively. The respiration rate of 144 h old ragi seedlings depleted by 0.86%, 8.76%, 54.2%, 67.1% and 100% inn MAC, LC_{10} , LC_{50} , LC_{90} and LC_{100} doses of lead nitrate in petriplate culture respectively. The gross primary production rate of 144 h old ragi seedlings depleted by 9.7%, 50.7%, 60.5% and 100% inn $LC_{10'}$ $LC_{50'}$ LC_{90} and LC_{100} doses of lead nitrate in petriplate culture respectively (Fig. D4). Residual lead was available in the roadside plants of the exposed area (both the sides of the freeways) and caused serious impact on the pigment content. The laboratory study also indicated severe effects of lead on the metabolic activity of the ragi seedlings in petriplate culture.



Discussion

The two main National Highways passing through Ganjam district was selected for survey. Both the roadside terrestrial plantations and different types of crop plants grown 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 Highways 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 gravish 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 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. The plants collected from the Karenjei area did not show any residual lead level in leaf tissues and least dust was deposited on the leaf surfaces 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. Out of 22 plant samples collected from the areas, only 10 plants showed significant accumulation of lead. The availability of lead in these plants was due to absorption of lead from air direct or from the deposition of lead with dust and other particulates, which were discharged from automobiles and the contaminated air by the vehicular exhausts reaching distances to contaminate the plants. Bazzaz et al., (1975) reported the existence of a significant relationship between lead exposure and depletion in photosynthesis of the plant as a whole and they presumed and accepted this to result from either coating on the leaf surface closing the pore and restricting the gaseous exchange or impact on guards cells leading to stomatal closure not a direct effect of lead in photosynthetic processes. Our data also agrees with the above finding as huge amount of dust deposit on the

leaf surfaces might be the cause of decrease in photosynthesis and also decrease in chlorophyll content. As these dust particles along with other chemicals form a smear layer over the leaf surface delimiting solar energy entry and closure of stomata. Kosobrukhov et al. (2004) suggested that the photosynthetic activity of plants is governed by many factors like leaf area, stomatal size, number etc. In addition the amount of chlorophyll present in the leaves is equally important for such activity. Burzynski (1987a, b) reported that lead can inhibit chlorophyll biosynthesis in plants by leading to impaired absorption and absorption of essential elements. Due to strong affinity of lead for protein Nand S- legands, the photosynthetic apparatus gets damaged (Ahmed and Tajmir-Riahi, 1993). Increased chlorophillase activity in lead exposed plant led to increased degradation of chlorophyll or destruction of chlorophyll pigment, which was responsible for the photosynthesis of plants (Drazkiewicz, 1994) by way of absorption of sunlight. It appears that lead might be one of the causative agents for decrease in chlorophyll content by way of degradation of chlorophyll and phaeophytin content. Significant depletion in carotene content was also equally important for determining the causes of impact of lead and other chemicals available in the air including dust coming from vehicular traffic. 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. In all exposed cases significant decrease in phaeophytin content was recorded when compared to their respective control value, except in Catharanthus roseus where an insignificant increase in phaeophytin to the tune of 1.6% was noted. Maximum decrease in phaeophytin content was recorded in case of Commelina benghalensis. Interestingly, in case of Tectona grandis, the phaeophytin content showed an insignificant depletion (3.4%) when compared to control leaf sample. 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 control leaf samples. Alterations in pigment content of the contaminated plants when compared to control plant leaf samples along with residual lead in the leaves. All the plants showed decrease in chlorophyll, phaeophytin and carotene 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 best reason can be insufficient residual lead load. The leaves of some plants turned brown 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. At MAC value increase in photosynthetic rate was recorded and at LC₁₀ onwards decrease in photosynthetic rate was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the photosynthetic rate decreased in 144h old ragi seedlings in petriplate culture. At MAC value instead of any increase, only decrease in respiration rate was recorded and at LC₁₀ dose onwards decrease in respiration rate was noted in lead nitrate exposed seedlings. At MAC value insignificant increase in gross primary production rate was recorded and after LC₁₀ value onwards decrease in gross primary production rate was noted in lead nitrate exposed seedlings. With the increase in lead nitrate doses the gross primary production rate decreased in 144h old ragi seedlings in petriplate culture under laboratory controlled conditions. The photosynthetic rate and gross primary production rate values increased at MAC value of lead nitrate in 144 h old ragi seedlings compared to their respective control values but increase was not statistically significant. Lead is a potent metal ion responsible for the inhibition of chloroplastic ATP synthetase / ATPase activity and for the destruction of the membranes (Tushu and Brouillette, 1987). Many authors reported that photophosphorylation is sensitive to heavy metal ions but no general agreement was reached pertaining to

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the mechanism of operation and the site of action. Bücker-Neto *et al.*, (2017) reported that aerobic reactions such as photosynthesis or respiration lead to ROS (hydroxyl, hydrogen peroxide or superoxide) production in plant cells and can damage and destroy lipids, DNA, proteins and important biological molecules (Noctor and Foyer, 1998). High concentrations of contaminants affect plants from molecular to physiological levels. However, the exact processes involved are not well understood and we agree with the observations of Bücker-Neto *et al.*, (2017). Higher studies will provide greater input to understand the atmospheric lead pollution and its impact on plant systems.

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