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IDENTIFICATION OF VEHICULAR EMISSION HYPER ACCUMULATOR AND ITS SUBSEQUENT USE AS PHYTOREMEDIATION

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Abstract

Effects of vehicular emission on two plants *Amaranthus spinosus* Linn. and *Croton bonplandianum* Baill, collected from polluted site and from relatively less polluted site/control site of Kolkata city of West Bengal, India was studied. Photosynthetic pigment content, proline content, phosphatase activity and Hill activity, heavy metal contents and their relevance to be used as phytoremediators was investigated. Decrease in pigment content and increase in proline content and phosphatase activity was observed in the plants from polluted areas. Micromorphological studies revealed the effect of automobile emission on epidermal cells and stomatal size on both the experimental plants. Data indicated that between the two plants, *A. spinosus* was more susceptible and *C. bonplandianum* was more tolerant to vehicular pollution.

Introduction

Vehicle exhausts as well as industrial emission contribute to air pollution due to incomplete combustion of carbon containing fuels which cause formation of various gases, liquids and solid particles. Although several physical and chemical techniques are available for environmental cleanup, huge cost, low efficiency, labour requirement and generation of harmful by-products hinder their applications. To overcome the disadvantages associated with the conventional methods, researches are being focussed on phytoremediation. Thus, plants provide one of the most effective and natural ways of removing atmospheric pollutants by absorption and accumulation of gaseous and particulate matter (Varshney 1985) and various plants have thus been explored for this purpose (Joshi *et al.* 1997, Ghafari *et al*. 2020).

Based on adsorption/absorption of air pollutants, plants have been categorized as sensitive, intermediate and moderately tolerant (Singh *et al.* 1991). While sensitive plants act as indicators, tolerant plants serve as sink to atmospheric pollutants. Generally, analysis of a combination of biochemical and physiological parameters are more effective in indicating pollution induced changes in plants than any individual parameter. Therefore, a number of factors like chlorophyll, carotene and xanthophyll content, Hill activity, proline content, acid phosphatase and alkaline phosphatase activity, of leaves as well as Pb, Cd, Co, Ni content of soil and plant materials were considered in this study. Here, these concepts were applied to study the impact of traffic related air pollutants on roadside vegetation of Kolkata city. In the present investigation, the impact of pollution on selected foliar micromorphological and biochemical parameters of *A. spinosus* and *C. bonplandianum* were evaluated. The present work was aimed at identifying the vehicular emission hyper accumulator plant among the two plants, in order to use them for phytoremediation.

Materials and Methods

Plants were sampled from either side of the highway, Barackpore Trunk Road, near Sinthee more, Cossipore, having close proximity to thermal power plants (polluted site) and from garden of Ballygunj Science College, a residential area (relatively less polluted site/control site) of

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Kolkata city of West Bengal, India. Two wild herbaceous dicotyledonous plants, *Amaramthus spinosus* Linn. and *Croton bonplandianum* Baill. were selected from these areas as preliminary survey of the area showed that the above plants have highest frequency and density at both sites. During sampling a completely randomized design with three replications was followed. The plants of the polluted and control areas at post flowering stage were brought to the laboratory for the investigation. For biochemical analysis, the leaves were washed with tap water and then with distilled water to dust free contamination. Soil samples and plant samples were collected from the same place in polluted and less polluted areas

Chlorophyll estimation was made according to Arnon (1949). Carotenoids were estimated according to the method of Davies (1976) with little modification. Hill activity was determined according to Vishniac (1957). Estimation of proline was made according to Bates *et al*. (1973). The acid phosphatase assay was done with p-nitrophenol as substrate, enzyme solution, acetate buffer (pH 4.8) and the reaction was terminated by NaOH. The colour intensity was recorded at 410 nm. Protein was estimated according to Lowry *et al*. (1951). Similar procedure was followed for alkaline phosphatase assay with sodium carbonate buffer (pH 10.0). Estimation of Pb, Cd, Co, Ni content of leaves and soil were done with the help of atomic absorption spectrophotometer at wavelength 283.3, 228.8, 240.7 and 232.0 nm, respectively. Acetylene and air were used as fuel and oxidant respectively. Micromorphological studies of control and polluted leaf surface architecture like stomatal frequency were studied. Each specimen was observed on Phillips Holland ^{P} SEM Model of Scanning Electron Microscope in suitable acceleration voltage, magnification and tilt.

Results and Discussion

In the two herbaceous plants under consideration, there is a consistent decrease in chlorophyll a (40.57% in *Amaranthus spinosus* and 34.37% in *Croton bonplandianum*), chlorophyll b (14.28% in *C. bonplandianum* and 6.9% in *A. spinosus*) and total chlorophyll content (30.87% in *A. spinosus* and 27.78% in *C. bonplandianum*) in the samples obtained from polluted site (Fig. 1). The photosynthetic pigments are fairly sensitive to air pollutants and reduction in chlorophyll content had been used as an indicator of air pollution (Pawar and Dubey 1985). Chlorophyll a showed a drastic reduction compared to chlorophyll b in both the plants studied. This observation is in close agreement with the work of Patidar *et al*. (2016). The decrease in pigment content might be due to the increase in leaf pH due to pollution that lead to pigment degradation or exerted an inhibitory effect on the activities of enzyme essential for pigment synthesis (Desai and Kapoor 2013). Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in photosynthesis and protect the chloroplast from photo destruction (Siefermann-Harms 1987). Decrease in carotenoid content was much higher than chlorophyll content (carotene content decreased in *A. spinosus* (76.57%), *C. bonplandianum* (45.24%). Xanthophyll content reduced by 59.2% in *A. spinosus* and 26.31% in *C. bonplandianum* from polluted site because they were more sensitive than chlorophyll and were found to decrease in response to pollutants (Chauhan 2010) (Fig. 2). Total average amount of assimilating pigments (chlorophyll + carotenoid) in the leaves of *A. spinosus* from polluted area showed more reduction compared to *C. bonplandianum* indicating that the plant species were under stress and pigment patterns were damaged due to pollution. Chlorophyll and carotenoid, both participate in photosynthetic reactions and are the central point of energy manifestation and thus depletion of pigments plays a significant role in inhibition of photosynthetic activity (Chauhan and Joshi 2008). Metals like Pb, Co, Cd and Ni are the major components of automobile exhaust and reports showed that chlorophyll and carotenoid content declined in presence of Co, Pb, Ni, Cd (Perez-Espinosa *et al.* 2002, Ghani *et al.* 2010, Dubey and Pandey 2011, Liu *et al.* 2014). These reports are in conformity with the present results.

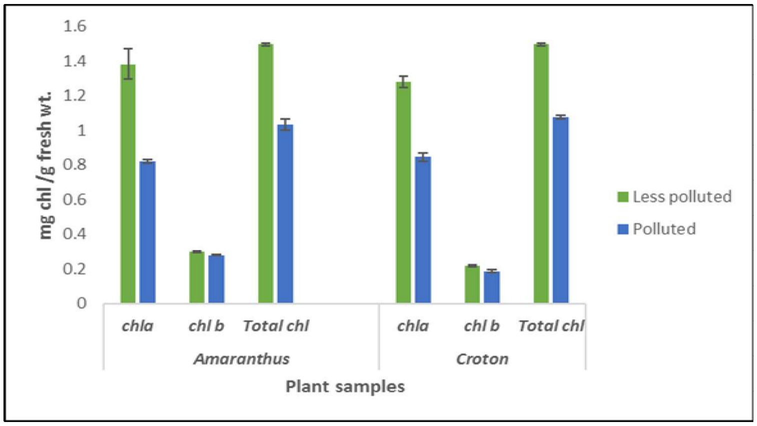

Fig. 1. Chlorophyll a, b, total chlorophyll content of *A. spinosus* and *C. bonplandianum* from polluted and relatively less polluted areas.

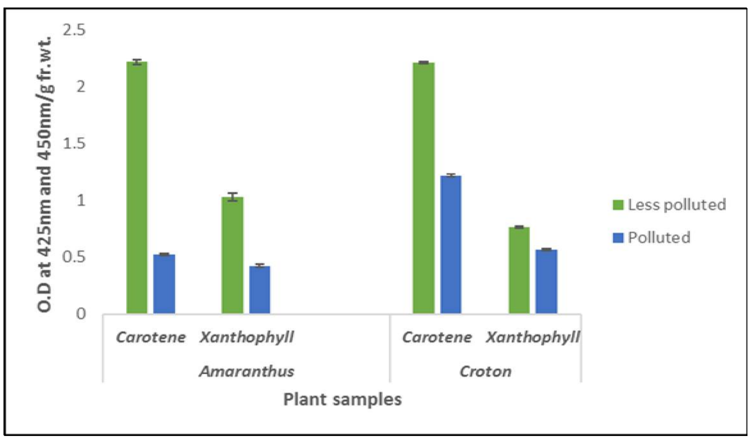

Fig. 2. Carotene and xanthophyll content of *A. spinosus* and *C. bonplandianum* from polluted and relatively less polluted areas.

Hill reaction is generally assumed to represent the photochemical splitting of water and consequent reduction of 2,6-dichlorophenolindophenol (DCPIP) by the reductant thus produced. DCPIP becomes colourless as it is reduced by intercepting electrons from the electron transport since as it has a higher affinity for electrons than ferredoxin. The photosynthetic electron transport chain can reduce DCPIP as a substitute for $NADP^+$ that is normally the final electron carrier in photosynthesis. In this context, the heavy metals present in the emission may be assumed to have caused impaired development and general depression of photosystem II reactions and O_2 evolving centers (50.54% decrease in *A. spinosus* and 27.66% in *C. bonplandianum*) (Fig. 3a). Earlier results reported by Mandal and Mukherji (2000), Sinha *et al.* (2002) in close agreement with the present findings. From the graph (Fig. 3b) it is apparent that total pigment concentration as well as Hill activity was greater in lesser polluted areas and irrespective to pollution levels in the areas, *C. bonplandianum* has higher Hill activity. It was observed that *A. spinosus* was affected more in terms of total pigment concentration than *C. bonplandianum* (fall in total pigment concentraion : 1.663 mg pigment/g fresh wt) and thus is more vulnerable to pollution. It was also apparent that Hill activity levels was affected more in *A. spinosus* (fall in Hill activity : 0.916 µ mole DCPIP reduced/h/mg chlorophyll) due to pollution than in *C. bonplandianum* (fall in Hill activity : 0.7 µ mole DCPIP reduced/h/mg chlorophyll). Greater reduction in total pigment concentration was found alongside greater reduction in Hill activity, so it can be inferred that Hill activity is dependent on total pigment concentration. It can also be finally inferred that *C. bonplandianum* is more pollution tolerant than *A. spinosus*.

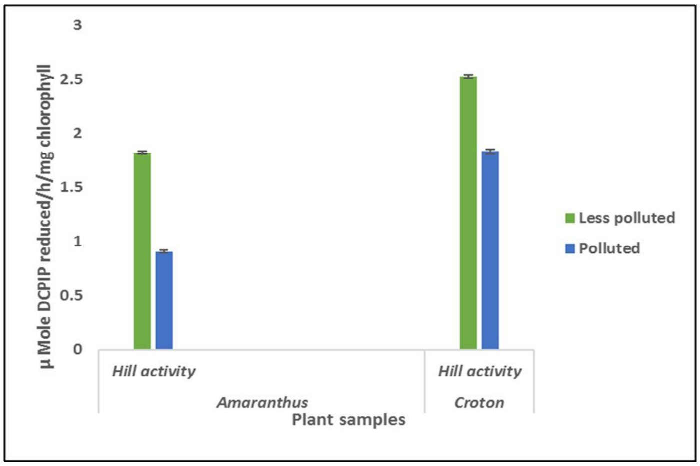

Fig. 3a. Hill activity of *A. spinosus* and *C. bonplandianum* from polluted and relatively less polluted areas.

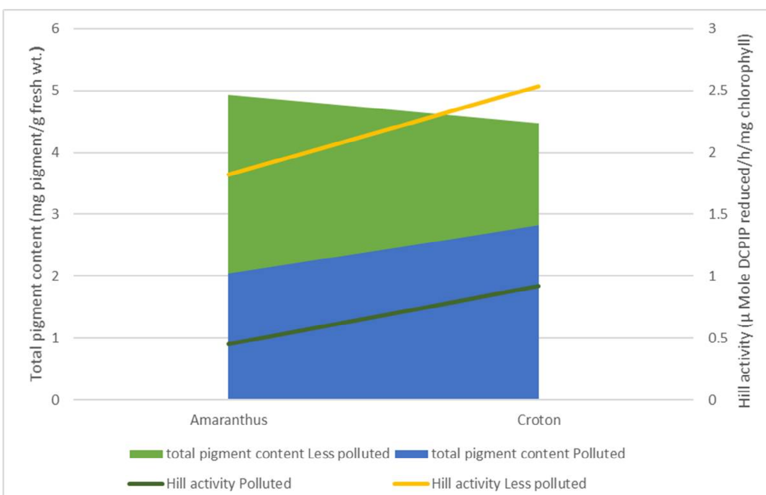

Fig. 3b. Comparative graph showing trends between Hill activity, total pigment content and Hill activity's dependency on total pigment content.

Proline is a universal osmolyte accumulated in response to several stress (Oncel *et al*. 1996) and may have a role in plant defense reactions (Khattab 2007). The two samples under investigation such as *A. spinosus* (44.35% proline content) and *C. bonplandianum* (73.54% proline content) showed a positive correlation between the proline accumulation and the level of pollution (Fig. 4). Similar results in *Peltophorum pterocarpum* (Desai and Kapoor 2013), *Thevetia*, *Magnifera*, *Psidium* (Patidar *et al*. 2016) were reported in response to air pollution. In the present experiment *C. bonplandianum* showed comparatively more increase in proline content, and so the plant is better adapted to combat pollution stress. This conclusion was further supported by Shankar and Jindal (2001), where they stated that proline was the important physiological factor in biochemical defense mechanism. Increased proline content can thus be used as a marker of vehicular pollution. An increase by 22.58 and 39.39% in acid phosphatase activity and 43.86

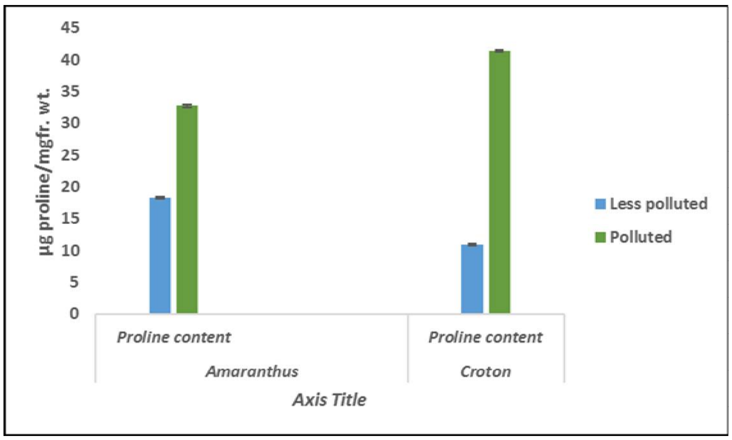

Fig. 4. Proline content of *A. spinosus* and *C. bonplandianum* from polluted and relatively less polluted areas.

and 28.86% increase in alkaline phosphatase activity was observed in *A. spinosus* and *C. bonplandianum* from polluted site. Thus it can be inferred that automobile exhaust had some stimulating effects on the acid and alkaline phosphatase activities of these plants (Fig. 5a, b). It is apparent from the graph (Fig. 6) that proline content, acid phosphatase and alkaline phosphatase levels were high in more polluted areas. Proline levels were higher in *C. bonplandianum* in more polluted areas. Alkaline phosphatase levels were found to be higher in *C. bonplandianum* whereas acid phosphatase levels were higher in *A. spinosus* irrespective of amount of pollution. Increase in alkaline phosphatase in *A. spinosus* (14.33 µg p-nitrophenol released/µg protein) was slightly higher than in *C. bonplandianum* (13 µg p-nitrophenol released/µg protein). Increase in acid phosphatase in *A. spinosus* (0.0876 µg p-nitrophenol released/µg protein) was slightly higher than in *C. bonplandianum* (0.0812 µg p-nitrophenol released/µg protein). Proline increase in *C. bonplandianum* (30.46 µg proline/mg fr wt) was very high as compared to *A. spinosus* (14.53 µg proline/mg fr wt). Thus considering proline the stress marker, it can be inferred from the graph that *C. bonplandianum* is more tolerant to pollution stress as compared to *A. spinosus*.

Analyses of soil and plant samples showed that the concentration of heavy metals e.g. lead, cadmium, cobalt and nickel were more near the highway. Lead concentration was 7 times greater in the soil samples of polluted area and hence is the most prominent among the studied heavy metals followed by cadmium (6 times), nickel (4 times) and cobalt (3 times). Similar trends of increasing metal content were found in the plants near the roadside. The contents of lead,

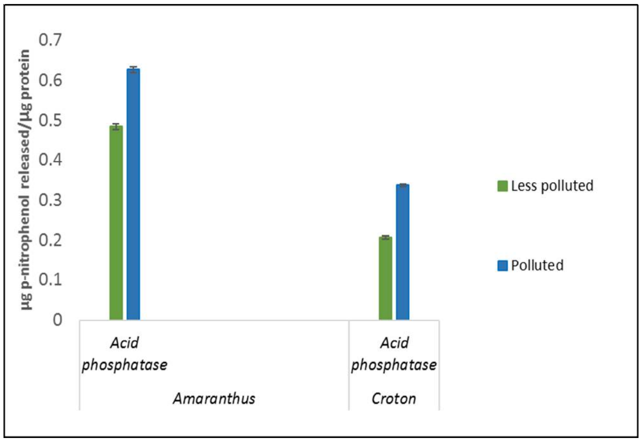

Fig. 5a. Acid phosphatase activity of *A. spinosus* and *C. bonplandianum* from polluted and relatively less polluted areas.

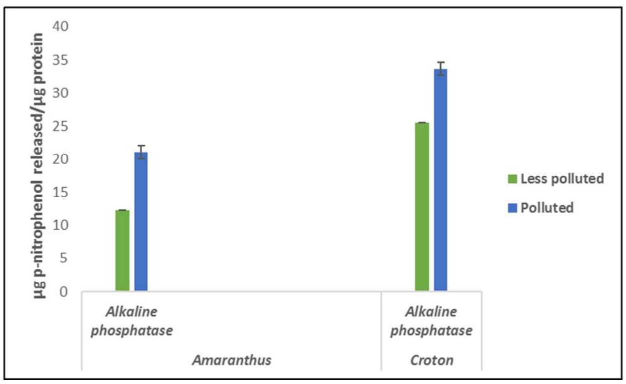

Fig. 5b. Alkaline phosphatase activity of *A. spinosus* and *C. bonplandianum* from polluted and relatively less polluted areas.

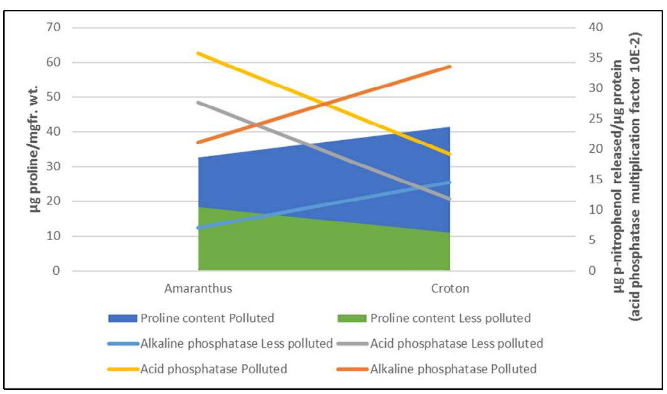

Fig. 6. Comparative graph of acid phosphatase, alkaline phosphatase and proline content (and their interrelationship) of *A. spinosus* and *C. bonplandianum* from heavily and less polluted areas.

cadmium, cobalt and nickel were significantly higher in the plants collected from more polluted area (Fig. 7a). Among the heavy metals lead content showed the most prominent difference between polluted and less polluted areas. Out of the two experimental plants, *C. bonplandianum* accumulated more heavy metals than *A. spinosus* and thus it may be inferred that *C. bonplandianum* was more likely to be the heavy metal accumulator among the two plants. Further from Fig. 7b it is apparent that heavily polluted soil had more heavy metals than less polluted soil sample as well as in both the plants from two experimental sites. It was observed that due to automobile emission there was an increase in the number of epidermal cells, consequent decrease

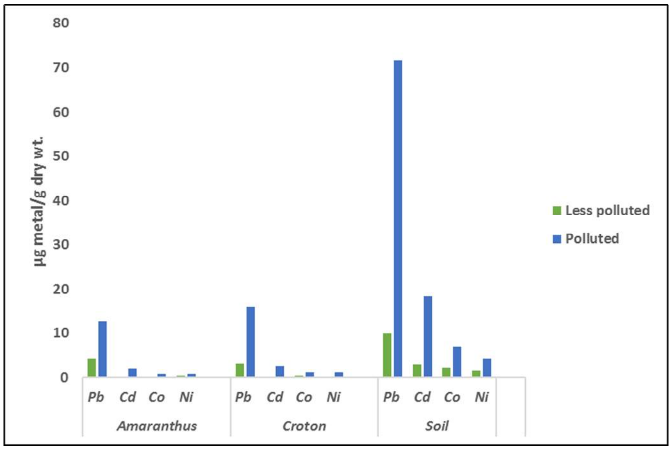

Fig. 7a. Lead, cadmium, cobalt and nickel content of *A. spinosus*, *C. bonplandianum* and soil samples from polluted compared to less polluted areas.

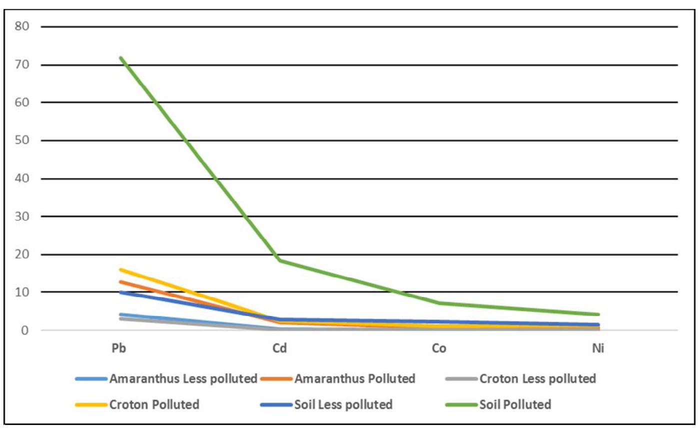

Fig. 7b. Comparison of lead, cadmium, cobalt and nickel content of *A. spinosus*, *C. bonplandianum* and soil samples from polluted and relatively less polluted areas.

in the epidermal cell size and decrease in the size of the stomatal aperture in the plants growing near the highway. Decrease in stomatal size and increased stomatal frequency may be an important strategy of avoidance of absorption of pollutants by plants (Gostin 2009). *C. bonplandianum* plants of the more polluted area showed epidermal striations along with a drastic decrease in the size of the stomatal aperture and increased stomatal density, indicating an adaptive measure of the plant against vehicular emission (Fig. 8). The high frequency of stomatal complex in the experimental plants might that it needed to transpire faster than normal to carry out biochemical activities (Ogunkunle *et al*. 2013).

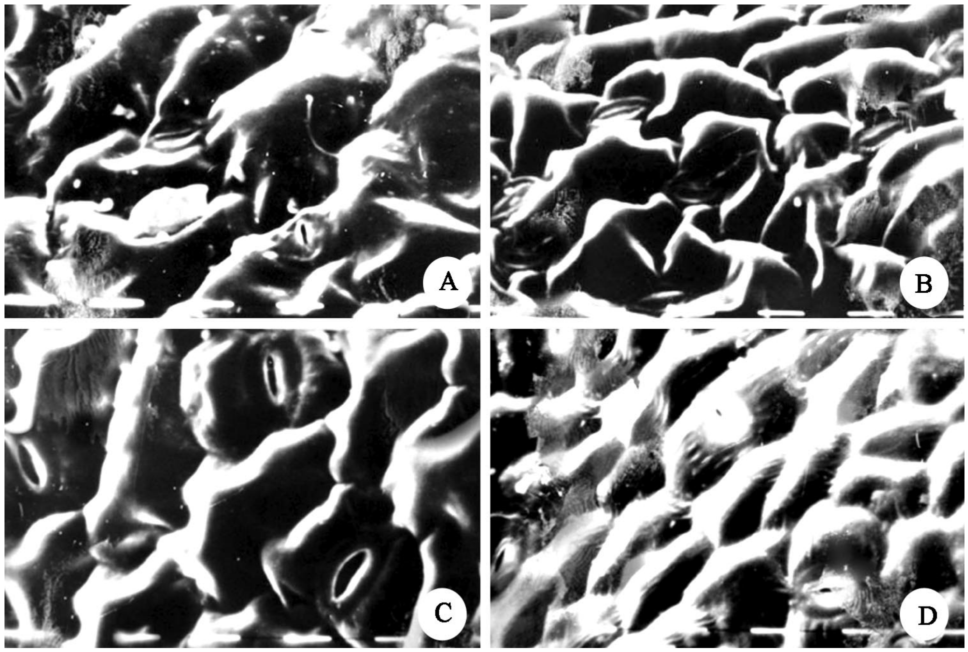

Fig. 8. Microphotographs taken from the dorsal surface of the leaves of *A. spinosus* (A), (B) and *C. bonplandianum* (C), (D) from relatively less polluted (A), (C) and more polluted (B), (D) areas.

Due to pollution increase, there was a steady decrease in chlorophyll content between the plants studied due to carotenoid depletion, which was known, to protect the chlorophyll pigments. In addition to this, Hill activity showed a sharp decline in both plants with increase in emissions. The stress indicators, namely proline, acid phosphatase and alkaline phosphatase all showed that increase in emissions have a positive correlation with pollution stress on the plants. The heavy metal content found in plants near the highway showed a sharp increase as compared to less polluted areas which indicated long time accumulation. It was also observed that among heavy metal accumulation, absorption of lead is comparatively high in both plants as the concentration of lead in plants of polluted areas, were more than the concentration in non-polluted soil. This is not seen for any other metal. However, the levels of changes found in both plants are different and thus lead to further conclusions. *A. spinosus* in polluted areas showed a higher decrease in chlorophyll a, b, and total chlorophyll content as well as the concentration of carotenoid pigments than *Croton* sp. in polluted areas. Also, the proline content (which is a known stress marker, an increase of which leads to better self-protection by plants) showed a higher increase in *C. bonplandianum* than in *A. spinosus*. Thus, it may be can concluded that *A. spinosus* is more susceptible to vehicular pollution than *C. bonplandianum* and *C. bonplandianum* was more tolerant to vehicular pollution and had better stress combating ability when exposed to emissions. Phytoremediation is a sought-after technique for cleaning up environmental contaminants in an economical and hazard free manner. "Hyper accumulator" plant species are characterised by their ability to absorb and accumulate high concentration of heavy metals in their shoots. Thus *C. bonplandianum* can be used as a hyperaccumulator and be planted near high pollution and emission zones to effectively cleanse the air and soil of heavy metal accumulation. *A. spinosus* being susceptible to pollution can be used as an indicator for vehicular emissions.

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