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Biophysical Characterization of Heavy Metal Stress in *Spirodela polyrhiza* (L.) Schleid

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Research Article

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Abstract

In the present investigation the effect of heavy metal stress on photosynthetic performance of *Spirodela polyrhiza* was studied. The photosynthetic activity was measured by using chlorophyll a fluorescence analysis. *S. polyrhiza* were treated with various concentrations of $HgCl_2$ (0, 0.2, 0.4 and 0.6 µM). After 48-hour, various fluorescence parameters (Fm. Fv/Fm and PIcsm) and chlorophyll a content were measured. Results show that with increasing the metal concentration, fluorescence parameters and chlorophyll a content were decreased. The study reveal that heavy metal decreased the photosynthetic activity of *S. polyrhiza*.

Keywords: *S. polyrhiza*; Fm; Fv/Fm; Chlorophyll a fluorescence; Performance index

Abbreviations: Fm: Maximum fluorescence; Fv/Fm: Quantum yield; PIcsm: Photosynthetic performance index per cross section; Chl *a*: Chlorophyll a; Hg: Mercury; PSII: Photosystem II

Introduction

In nature plants are contentiously faced with biotic and abiotic stresses. Through industrialization and other anthropogenic activities heavy metals such as, Hg, Cu, Zn, Pb, Cd, Co, Ni, Fe, As etc. are introduced into environment [1]. In plants heavy metal stress disturb function of important enzymes and proteins. Heavy metal stress also interferes with various physiological and metabolic functions of plants such as photosynthesis, respiration etc. [2]. On the other hand, increased level of heavy metals enhance the reactive oxygen species amount which trigger oxidative stress [3]. Mercury (Hg) is a highly toxic element for the plants without any beneficiary effect [4] and it is five to ten time more toxic than Cu [5]. The decrement in Chlorophyll and other pigments is one of the major effect of heavy metal stress [6]. At multiple sites including the OEC, the PS II reaction center and the antenna of PSII, Mercury hinders photosynthesis electron transport [7].

Chlorophyll *a* fluorescence is an important tool to study the effects of various environmental stresses on photosynthesis [8]. It is a key approach to analysing PSII function and its response to environmental changes and other growth conditions [9].

Duckweeds is a group of small aquatic plant species found in all around the globe and mainly reproduces by vegetative budding found on frond [10]. Duckweed is monocotyledon and placed in family Lemnaceae [10]. The Lemnaceae family comprises four genera, Lemna, Spirodela, Woiffia, and Wolffiella [11]. Duckweeds have a great ability to tolerate adverse condition. Therefore, species having high potential for phytoremediation [12].

Some chlorophyll parameters such as Fv/Fm, Fm and PIcsm and Chlorophyll a content was analysed in the

Spirodela polyrhiza grow under various concentration Hg.

Materials and Methods

S. polyrhiza used in this investigation were collected from a freshwater pond in Udaipur City, India. The fronds were acclimatized in 10 % Hoagland's growth medium in laboratory condition as per OECD 221(6500–10000 lux light irradiance, 14-h photoperiod, and 25/20°C day/night temperature [12]. After 1 week of acclimatization of plants in medium, healthy, similar-sized fronds (about 3 g) were treated with Hg^{+2} treatment was induced by incorporating the various concentrations of $HgCl_2$ (0, 0.2, 0.4, 0.6 µM) in the medium (Figure 1).

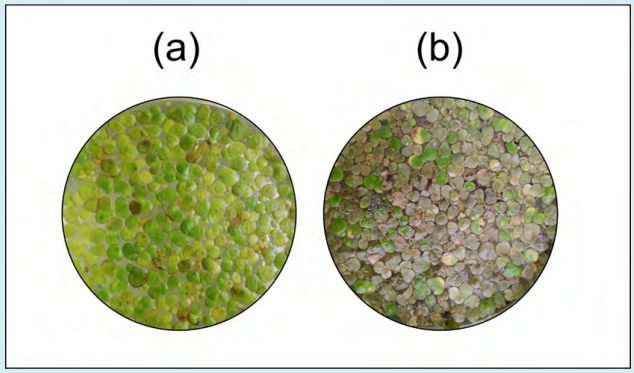

Figure 1: Fronds of *S. polyrhiza* under normal (a) and Hg stress (b) condition.

Chlorophyll a fluorescence was measured using plant efficiency analyser (Handy PEA fluorimeter, Hansatech instruments Ltd. England) after 48 hrs. of heavy metal treatment. Before measurement duckweed fronds were dark-adapted for 50–60 min at 26°C. Thereafter, Chl a fluorescence signals were analysed with the Biolyzer v.3.0.6 software (developed by Laboratory of Bioenergetics, University of Geneva, Switzerland) [13]. Appropriate numbers of replicates were taken and the experiment was repeated three times to ensure the results. Abbreviations, formulas, and definitions of the JIP-test parameters used in the current study are Fv/Fm, Fm and PIcsm.

Results and Discussion

Chlorophyll fluorescence often represents the function of photosynthetic complex [14,15]. A decline in PSII quantum yield represented by Fv/Fm was observed during the heavy metal treatments at successive concentrations. When plants grow under a favorable environment, Fv/Fm is kept in a stable range but it decreases, under the adverse environment [16].

The rapid decline in Fm (Figure 2a) would suggest a change in the ultrastructure of the thylakoid membrane, affecting the electron transport rate. The reduction in PSII photochemical efficiency could be partially attributed to the destruction of antennae pigments [17]. With the decline in quantum yield and, therefore, reduction in electron transport rate, a gradual increase in the pH will become established across the thylakoid membrane, as a result of the heavy metal impact on the photosynthetic apparatus. Maximum fluorescence, defines the maximum number of reaction centers reduced or closed by a saturating light pulse. In general, the greater the plant stress, the fewer open reaction centers available, and the Fv/Fm ratio is lowered [18]. Maximum Fluorescence level decreased with the increase in Hg concentration and found to be minimum in case of 0.6 µM Hg, 0.4 and 0.6 µM Hg concentration almost showed the same pattern.

Quantum yield (Fv/Fm) decreased with increase of Hg concentration having no significant difference between the 0.4 and 0.6 treatments but with comparatively lower values from control (Figure 2b). Exposure of *S. polyrhiza* to Hg concentration decreased the Fv/Fm ratios, which characterize the functional activity of the photosynthetic apparatus. The inactive reaction center of PSII that can result in photoinhibition might have resulted in lower Fv/Fm under different concertation [19].

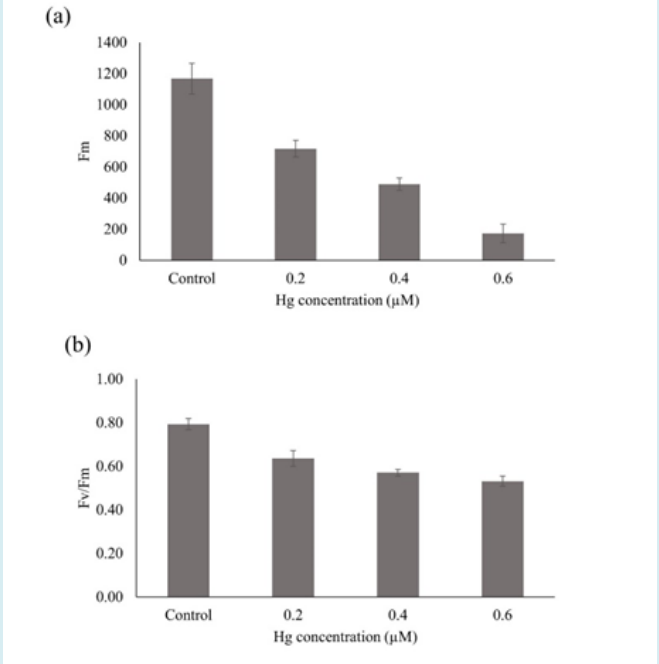

Figure 2: Effect of Hg stress on maximum fluorescence (Fm)(a) and quantum yield (Fv/Fm) ;(b).

Photosynthesis performance rate of the *S. polyrhiza* fronds decreased rapidly with the successive Hg concentration but it is minimum in plants treated with 0.6 µM Hg as compared to control (Figure 3a). Performance index (PI) is considered to be a good indicator of stress, which is the combined measurement of the density of RC, maximum energy flux reaching PSII reaction centres and the electron transport. The significant decreases in PI in heavy metal stress reflect an inefficient performance of PSII [20]. Our experiment showed that chlorophyll a content was reduced with successive concertation (Figure 3b), which was primarily caused by fast reduction in the reaction centers than LHC of PSII. Changes in Chl a content are also considered an indicator for relative photosystem stoichiometry that help us determine the changes in the size of the light-harvesting antenna of PSII and the PSII amount [21].

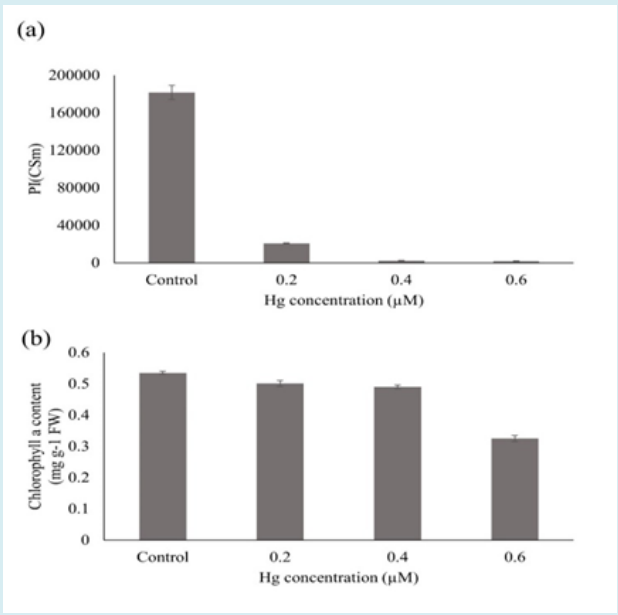

Figure 3: Effect of Hg stress on performance index (PIcsm); (a) and chlorophyll a content (b).

Conclusions

In conclusion, Heavy metal decreased the florescence yield due to restrictions of electron flow at oxidizing side of PS II. Increasing concentrations of Hg decreased the photochemical efficiency (Fv/Fm), photosynthetic performance index (PIcsm) and Chlorophyll a concentration. Thus, Hg stress significantly inhibit the photosynthetic function of *S. polyrhiza*.

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