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Journal Name:	British Journal of Applied Science & Technology
Manuscript Number:	2013_BJAST_6767
Title of the Manuscript:	Antioxidative Response of <i>Hydrilla verticillata</i> (L.f.) Royle under Short Term Exposure to Mercuric Chloride
Type of the Article	

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	The focus of this study is to provide information about the response of <i>Hydrilla verticillata</i> in a short term exposure to different doses of mercuric chloride. Moreover, the effect of ascorbate in the restoration of chlorophyll loss were evaluated. Overall, the experimental methods seem sound, with some interesting results, but in some places the methods are too shallow and do not provide adequate details to evaluate the work. Major revision: 1) It is unclear from the text how much (gr, mg, etc.) tissue was used for the extraction and estimation of total chlorophyll contents, soluble proteins, ascorbic acid and MDA. Moreover for extracts preparation and the assay of enzyme activities there is not indication, in the paper, about the quantity of tissue used per volume of buffer. 2) In the Results section (rows 162-166) Authors stated that the addition of ascorbate in the medium allows a recover in the chlorophyll contents but the numbers reported in table 1 say that with 1mM	At the out set, on behalf of all the authors I would like to thank the anonymous esteemed Reviewer for reviewing paper in detail and giving his valuable observations and suggestions. 1. Since the vaues are expressed in terms of g/FW for all the parameters, specifically the amount of tissues taken for different parameters have not been mentioned separately. However, the amount of tissues taken for all the parameters was 200 mg each for different samples and the volume of extract prepared in each case was 5 ml (for MDA and soluble protein the volumes of extracts are mentioned separately below), including in case of enzyme extracts also. But in case of MDA extraction, 200 mg of tissue was homogenised with 5% TCA and the volume of extract was made to 1.0 ml and



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	ascorbate there was a drop in chlorophyll content. 3) Check whether the sentence “<i>Hydrilla verticillata</i> twigs” (row 166) is in the right position.	the entire extract was taken for estimation of the MDA content following due procedure. In case of soluble protein, 200 mg of tissue was homogenised with buffer and the volume was made to 5.0 ml after centrifugation, 1.0 ml of supernatant was mixed with 1.0 ml of 20% TCA for protein precipitation. After washing the precipitates with different reagents (detail mentioned in the MS), the pellets were solubilised with 2.0 ml of 0.3 N NaOH and from that, 0.5 ml was taken for soluble protein estimation. 2. In fact, the value of total Chl content for the sample HgCl₂ (50 mg/L) + Ascorbate (1 mM) is 1.196 ± 0.136. It was erroneously typed as 0.906 ± 0.07. I am extremely sorry for such error. 3. No. It is not in the right position. Virtually it is not required there and has already been deleted in the revised MS.
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Minor REVISION comments	1) Row 173 check the value reported and the text alignment. 2) Figs. 2, 3, 4. Please, explain the meaning of the double and triple letter above the bars. 3) Discussion. Maybe the recent work by Michelet L. et al. (Down-regulation of catalase activity allows transient accumulation of a hydrogen peroxide signal in <i>Chlamydomonas reinhardtii</i>.” Plant, Cell and Environment (2013) 36, 1204–1213) could be useful to explain light effects on catalase activity. 4) Possibly, to measure the enzymatic activities after the treatment with ascorbate would make the work more complete.	1. Value has been checked and corrected as mentioned in the point 2 above. In the revised MS, the corrected value is in the line 181. 2. Fig. 2 and 3 are now mentioned as Fig. 1 B and 1 C respectively and Fig. 4 is now mentioned as Fig. 2 in the revised MS. In these Figures, the parameters whose differences are statistically significant from the others are marked with different letters on the vertical bar. But, if the difference of a parameter of certain sample is not significant from its neighbouring sample, then in that case the vertical bar has been marked with two or three letters, as the case may be. For example in Fig. 1 B (the empty box series, □), the samples are marked as 1 – a, 2 – ab, 3 – bc, 4 – d and 5 – e. This indicates that the difference in the sample 2 is statistically not significant from sample 1 (since both are marked with ‘a’) as well as from the sample 3 (since both are marked with ‘b’). But this sample (i.e. 2) is significantly different from other samples like 4 and 5. Similarly the sample 3 is not significantly different from 2 (since both are marked with ‘b’), but it is significantly different from the samples 1, 4 and 5. Similar is the case in case of Fig. 1 C (i.e., Fig. 2 in original MS). In Fig. 2 C (i.e., Fig. 4 C in the original MS), there is one three letter marking (abc) on the vertical bar of one sample (i.e., sample 5 of closed box series, ■). This indicates that the difference in this sample is not statistically significant from other 4 samples of the series.
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		3. As suggested, the findings of Michelet et al. (2013) has been incorporated in discussion part (Line 340-344) of the revised MS), while discussing the effect of light in declining the catalase activity under Hg stress. 4. No doubt, assessment of antioxidative enzyme activities after ascorbate treatment would have made the experiment more complete. But in some earlier study, loss of chlorophyll has been taken as an indication of prevalence of oxidative stress situation in plants (Shaw, 1995; reference already there in the MS). Therefore, measurement of total Chlorophyll only has been done in the samples treated with ascorbate in presence of mercury in order to assess the protection of oxidative injury by ascorbate on the basis of gain in the total chlorophyll content. Since, ascorbate was found to reduce the mercury induced chlorophyll loss; it is presumed that probably ascorbate has given protection against oxidative injury.
<u>Optional/General</u> comments	This manuscript is understandable but English grammar and word usage need attention before it can be published.	During this revision, some changes in sentences as well as English grammar have been done in revised MS. If some errors are left, these may be brought to our notice for further rectification.