



SDI Review Form 1.6

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

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

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 present work assessed the <i>Hydrilla verticillata</i> twigs response to increasing Hg stress in term of metabolites and enzymes activities changes after short treatment duration (3 h). As authors indicated in their manuscript, similar studies were undertaken on <i>Hydrilla verticillata</i> responses to heavy metals stress, including Hg. Here, they can found same comments that may improve their submitted work: - line 166: <i>Hydrilla verticillata</i> twigs ? deleted or complete the sentence. - Table 1: Verify the significance of difference between chlorophyll content in HgCl₂ (1.05c ± 0.141) and in HgCl₂ + Ascorbate (1 mM) (0.906d ± 0.07) ? - Subsequent results : Why effect of ascorbate addition on the other parameters (Soluble protein, enzymes activities, MDA) was not presented as for chlorophyll content ? - In the paragraph lines 179-381: "In presence of light there was 42% and under dark there was 180 26%	On behalf of all the authors, I would like to thank the esteemed anonymous reviewer for thoroughly reviewing the paper and giving valuable suggestions for the improvement of the quality of the paper. As per suggestions, modifications have been done in the text. -This has been deleted and the sentence has been completed. -In fact the value of Chlorophyll of the sample "HgCl₂ + Ascorbate (1 mM)" is 1.196^a ± 0.136, which was erroneously mentioned as 0.906 ± 0.07 in original MS. It is now corrected in the revised MS. - No doubt, measurement of soluble protein content, lipid peroxidation level along with the 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



SDI Review Form 1.6

	decrease in soluble protein content.....": Yu must precise the Hg concentration inducing theses changes (i.e: the severe stress, 200 ppm..?) - I advice authors to combine Fig 1, Fig 2 and Fig 3 into one single graph Fig 1: (A) total chlorophyll, (B) sluble protein), (C) Ascorbic acid. Then you cite them as Fig 1A, Fig 1B, Fig 1C. You do the same for enzymes activities (Fig 2A, B, C). - Line 227: the sentence "was measured assess": is not clear, may be you should delete "measured" - Fig 4: significance of differences for Cat and POX activities should be revised (see the control a,b ? 50 ppm ?...). - Discussion: this part contains a lot of literature without a focalized results discussion (relation between parameters changes). I suggest that author give more explanation about changes related to light/dark situation in relation to photosynthetic events (Photoinactivation mecanisms, electron transport chain disturbances, increasing catabolism..). - Conclusion: Lines 378-379: "Since only 3 h of exposure to Hg, the plant responded severely, exposure to a longer duration would have	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. -In line 207 of the revised MS, 200 ppm HgCl₂ has been mentioned as "severe stress". -As suggested, Fig. 1, Fig. 2, and Fig. 3 have been assembled in one Fig., i.e., Fig. 1 and accordingly mentioned through out the text. Fig. 4 and Fig. 5 of original MS have now been changed to Fig. 2 and Fig. 3 respectively in the revised MS and accordingly modified through out the text. -In fact 'to' should be there in between 'measured assess'. This has now been corrected in the revised MS in the line 230 and it now reads as 'was measured to assess'. -In Fig. 4 (which is now Fig. 2 in revised MS), significance level of difference has been done
--	--	---



SDI Review Form 1.6

	catastrophic consequence." : I don't agree with authors, Hydrilla twigs may display adaptive mechanisms and survive, you should to assay a long term Hg stress to affirm you conclusion. - Lines 379-381: "However, application of antioxidant (e.g., ascorbate) along with Hg has added significant relief to the toxic effects.": You don't have sufficient data to give such affirmative conclusion: you have just assayed ascorbate on one parameter (chlorophyll)? - Same references should be updated, there was only one recent reference (2012).	within one series of data, i.e., between the catalase or peroxidase values at different concentrations of the samples either exposed to light or kept under dark. Comparison has not been done in between the values of light and dark samples. In case of catalase, control light and dark values have been marked as 'b' and 'a' which does not mean that these two values are significantly different from each other. Comparison has been done between light control, light 10, light 50, light 100 and light 200. Similarly, dark control has been compared with dark 10, dark 50, dark 100 and dark 200. - In the discussion part, some literature has been given only to correlate the significance of findings and in my opinion these sentences have made the discussion more meaningful. Secondly, in fact photoinactivation, electron transport chain disturbances etc. happen in chloroplast under excessive irradiation. But that is a different chapter of study and we have not emphasized on that aspect in this study. Here, emphasis has been given on the effect of Hg on the antioxidative efficiency, under two conditions, i.e., light and dark. Therefore, in discussion emphasis on the phenomenon like photoinhibition etc. has not been given and antioxidative aspect has much highlighted. - "Since only 3 h of exposure to Hg, the plant
--	--	--



SDI Review Form 1.6

		responded severely, exposure to a longer duration would have catastrophic consequence." This is just an apprehension under severe case of mercury toxicity. No doubt longer period of exposure would have given sufficient evidence to such apprehension. But when only three hour of exposure has resulted in 65% loss in total chlorophyll and the bleaching was visually noticeable (mentioned in the first para of result), we did not feel the necessity of increasing exposure duration. Further, 65% loss in total chlorophyll can be considered as "plastic strain" and when the strain reaches plastic stage, there is no chance of reverting to original condition and adapting to the stress situation. Secondly, Gupta and Chandra (1996) have exposed the Hydrilla to 5.0 μM concentration of Hg, but in our case we have started from 36.8 μM (10 mg/L) on wards which is quite high. Therefore, longer period of exposure, beyond 3 h, has not been done. -“Application of Ascorbate has given significant relief” – this has been assumed on the basis of gain in total chlorophyll content in Hg treated twigs. Of course, assessment of other parameters in ascorbate treated twigs would have substantiated it, but since chlorophyll loss has been considered as a indication of prevalence of oxidative stress in plants in other reports (Shaw, 1995), such an opinion has been given. Please refer to the point 3 of the explanation above. Explanation in this regard has also been given there. -Three new references have been inserted out of which one is of 2000 [5], second one is of 2004 [4] and third one is of 2013 [30].
--	--	--



SDI Review Form 1.6

<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		