

In the manuscript entitled: Effects of substrate and water depth of a eutrophic pond on the physiological status of *Vallisneria natans*, a useful aquatic plant for water environment restoration in urban areas” authors explore the limiting factor for the growth of *V. natans* in eutrophic urban ponds. They measure various compounds in root and shoot to assess the stress level of *V. natans* when grown under different depth/light condition and substrates.

### General comments

- 1- The title can be more concise. Try removing the second part: a useful aquatic plant...
- 2- The study is conducted using a small laboratory experiment and then in the field using a eutrophic urban pond. But results and discussion on these two parts of the study are not well organized. The text needs to be reworked and reorganized to clarify what authors have found from each experiment separately and then what the pilot study (lab exp) contributed to the field study.
- 3- Tables and figures are not consistent. They are not clear and lack information necessary for the assessment of research findings. Most importantly, statistical analysis needs to be incorporated into the figures or shown to clearly demonstrate the differences across depths and substrates.
- 4- Statistical analysis is not explained properly. With two experiments (lab and field) and different experimental designs, authors need to provide a complete explanation of how and why they decided to run the stats with the current method.
- 5- Several compounds are measured and discussed in the text based on leaf analysis and the majority of the results and discussion are focused on these compounds without a word of how the plants actually performed at different treatments/depths. only plant assessment for lab exp was shown which seem unrealistic. The data are perhaps rounded up and somehow all std dev are 5! In addition, there is no description on how the authors assessed these data (height, fresh weight, etc.)
- 6- Substrate is a big component of this study and it is even in the title. But there is no information on substrate texture and chemistry.

I recommend rejection of the manuscript. This manuscript can be significantly improved by English editing and careful attention to the disclosed comments.

### Abstract

20 it is better to define “physiological status” or be more specific. Please state what physiological traits you have measured.

22 “water environmental conditions” sounds odd. Throughout the text.

26 “different physiological status” is vague. You could change the sentence to” MDA, SOD, CAT and POD content differed in leaves and roots when plants were grown in different substrates...”.

35 due to environmental stress. This is too general. If your treatments are depths, just make statements about the depth to avoid generalization.

34-38 needs reorganizing to make it clear what is compared to aquaria or sand. Try to separate the findings between the laboratory and field experiment to avoid confusion.

- 40 bottom mud has adverse effect?! Why is that expected? Please explain.
- 42 this study talks about water depth and ties that with light attenuation. But there is no mention to light intensity at different depths in the abstract.
- 53 please avoid using the word “purify”. Plants do not purify the water.
- 63 “because of less survival of planted individuals” sounds odd. Maybe change to ‘ because of low survival rate of transplants or introduced plants”
- 64 inhibits
- 69 shorten the sentences, here: . but it is not clear whether...
- 70-72 I do not understand the connection between hydraulic forces of waves in large water bodies and lakes to this study on small urban ponds. I suggest removing this statement.
- 75-76 you measured both Chl a and b. explain their difference here and what different levels of each means.
- 77 isnt it better to say photosynthetic ability?!
- 79 you should state that ROS induce lipid peroxidation. Otherwise there is no connection between the two sentences.
- 80 please explain why under stress conditions, production of SOD, CAT and POD is increased but their activity (line 83) is reduced.
- 97 remove “ of the park”. The pond is approximately 300 m long...
- 99-101 I recommend moving these statements about permits to the acknowledgement sections.
- 105-106 “... leaves on the water surface have shown an obvious black color in recent years.” I do not understand what the authors are trying to convey here.
- 108 remove “a location in”.
- 102-110 in this paragraph, authors could simply say that, in the study site/pond the restoration of *V. natans* has failed repeatedly despite... list all the failed trials including aerator installation.
- 111-117 how were these measured? Using what device/probes?
- 118 black color and anaerobic odor? Is this all we know about the collected sediment/mud?
- 136-138 The two substrates are being called different things throughout the text. Here is the longest name used have in the text: Bottom mud of the pond and River coarse sand. I suggest calling them simply mud and sand, and at first mention describe how you collected the mud (from the bottom of the pond) and sand. Also provide the chemical and physical properties of both substrates.
- 141 replace “allayed” with placed.
- 142 three different depths... what are the depths.

145-148 do you need all these (Temperature, pH, DO, ORP, EC, Turbidity, Chl a (what about Chl b?)) parameters? If you do not discuss them, you should remove them from the text.

153 remove “during the experiment”

153-155 other researchers do not need to know about the permitting agency. Move these to the acknowledgement section.

159-164 rephrase all these sentences. Do not start explaining each method with “By...”. For example, digested water samples were analyzed for TP using molybdenum...

172 “converted to mg g<sup>-1</sup> of fresh leaf.” Also how did you measure the fresh weight? Did you ensure that there is no water on the plant tissue when weighting them? How did you deal with the clonal growth? Did you remove them? Are new clonal growth a part of the fresh weight measurement?

193-194 please expand on this statement.

205 statistical analysis

I suggest separating the statistical analysis into two sections and explain the design for lab and field experiments separately. In the result section, you explain the split plot design first but in the stats section, you described it in the latter section. These need to be consistent.

216 was there a depth factor in the aquaria design? I am getting confused.

224 are these reported numbers for both substrates?

227 report nitrate conc at the beginning and at the end.

229 nutrients? Or are you still talking about nitrate? Also, reduction in nutrient may indicate nutrient uptake but not plant growth. Please remove the statement about growth.

232 heterotrophic microbes... please provide supporting literature.

235 change to... was almost double than the growth in sand...

237 is there a statistical analysis done on this part to detect significant differences between the growth of *V. natans* in the two different substrates?

238-242 start this section by stating that there was a temporal difference between Chl a... content of the leaves and then point at the increased levels by reporting the concentration at the specific days into the experiment.

242 if statistical analysis was done on these measurements, why they are not incorporated in the figures?

246 if both Chl a and b had the same trend, you could revise this paragraph to reflect this observation. That would be much more effective than making the reader go through them one by one.

248 MDA increased by 5 or 10 d in both leaves and roots. What does this mean?

258 the difference was small? Table 3 shows that there is No difference!

266 how much was the decline? 10%? 50%? Is this a statistically significant decline?

271-274 why are these important? Did temperature and rain both had a significant effect on the experiment? Daily rain and temperature have equal effect (if any) on the treatments. If this is the case,

then reporting them does not add to this manuscript and I suggest removing all the statements and fig/tables related to temp and rain.

275 all the water equality parameters changed by day? Did this fluctuation have a sig effect on the treatments? What do you mean by ...turbidity were recorded at 0 or 5 d. ? does it mean that they were recorded on 5-day intervals?

335 as mentioned before, the statistical analysis was not shown and it needs to be incorporated in figures or authors provide a table to show there was a statistically significant different between Chl level in leaves in mud than sand.

What was found in the laboratory experiment?

345 what is the hypothesis behind the mud having an adverse effect on the growth of v. natans?

350 I suggest saying that the light quantum or light penetration was reduced by 50% and 90% at depths...

355 sharply increased by 10 d? please mention the fig number. I do not understand what authors are trying to convey here.

359 please cite literature that also reported similar observation under low light conditions instead of chemical exposure. Also what does severe chemical conditions mean?

389 what is the day of peak activity? Please explain?

401-404 this is interesting but should move to the discussion section where you can cite the paper by Han and Cui and discuss their finding. Also this needs to be further discussed since you had a treatment at 0.5 m depth so explain how your treatment performed under this condition.

408 I do not recall where in the text you showed a correlation between rainfall and nutrient concentration! This needs to be removed.

Fig1 mud of pond bottom. Please change to "collected mud".

Fig2 if these cages were used. Im afraid they might have blocked quite a lot of sunlight themselves!

Fig 3 what is d? please specify how many observation/data point is shown by each circle.

Table 1 the table and its caption does not indicate what each column represents. For example, what are the two "after"? also state the duration of the experiment.

Table 2 how are these measurements done? The numbers seem too clean and all appear to be rounded up. This table is for the lab experiment. What about the field experiment? Did the plants survive 30 day in that depth? How was the growth and other growth components?

Table 3 why was beaker/tray number the random factor and was not considered a replication? You have df for chl a/b in plant organs? This was not measured and there is no statistical analysis on it. Please leave it blank.

Table 4 is confusing. You have different parameters measured on 5-day interval, but at what depth? Then you have different depths that numbers do not differ by much! Should you replace them with a

range to simplify the table? The significant difference for depth at the last column, is comparing the three depths (0.5, 1.2 and 2 m)?

Why transparency is not measured at different depths? Secchi disk readings could be the most important measurement in this study since you are focusing only on water depth in the field experiment.

What is the + for the TP p value?

All the water quality parameters, those reported in mg/l are not different across depths. why report them for different depths?