

# Review of: "Phytotoxicity of atrazine combined with cadmium on photosynthetic apparatus of the emergent plant species *Iris pseudacorus*"

Dinggui Luo<sup>1</sup>

<sup>1</sup> Guangzhou University

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This research investigated the phytotoxicity of atrazine combined with cadmium on photosynthetic apparatus of the emergent plant species *Iris pseudacorus*, and further explained the toxicity mechanism of atrazine on the photosynthetic path and the alleviating effect of the cadmium by the continuous excitation chlorophyll fluorescence kinetic analysis. The work is meaningful due to concerning the joint effects of pesticides and heavy metals on non-target aquatic plants. However, the concentration of cadmium using in this study is high which should be clarified if it is common in the growing environment of *Iris pseudacorus*. And some problems still exist in this article and need to be answered and corrected. These problems are listed below.

Before pointing out the problems of each section, three common problems in the full text should be noted: (1) some JIP-test parameters didn't use the subscript. Please correct the form of these parameters in the full text; (2) all the abbreviations must be defined at their first mention. For example, what's the meaning of  $P_{680}$  and RCII? General readers may not understand the abbreviation of the terminologies at the first reading; (3) the author should supply the result of significant differences between the treatments in the Results section. For example, in the section of the K-band illustration, the  $F_k/F_j$  and  $W_k$  were compared between the single ATZ treatment and the combined treatment (ATZ above 1.0 mg/L) while the result of significant differences was not shown in Figure 5. I suggest that the comparison result can be listed in the supplementary information.

## Introduction

1. This article aims to clarify the phytotoxicity of atrazine combined with cadmium on *Iris pseudacorus*, however the author only illustrated the risk of Cd on human health. I suggest the author can add some cases about the hazard effect of Cd on aquatic plants.

2. Sentence:

Yet, none of these investigations addressed the joint toxicity of these contaminant mixtures to aquatic plants which are of great importance in stabilizing **function** and structure of freshwater ecosystems.

Comment: Add "the" before "function".

## 3. Sentence:

Therefore, there is a clear need to **focused** on the combined effects of ATZ and Cd on aquatic plants.

Comment: There is an incorrect form of the verb “focused”, please correct it.

4. What are the advantages of the chlorophyll fluorescence kinetics as the indicator of photosynthesis capacity in this work? Please briefly state in the section of Introduction.

## 5. Sentence:

Moreover, photosynthesis is the basic **physico-chemical** process for plant growth and survival, and is usually considered as a top priority in probing **toxicological** action of a compound toward plants (Gao et al. 2018).

Comment: Replace “physico-chemical” as “physical and chemical”, and add “the” before “toxicological”.

## 6. Sentence:

Thus, **photosynthetic** characteristics of plants can provide more direct and fundamental **evidences** for joint action induced by these two photosynthesis inhibitors.

Comment: Add “the” before “photosynthetic”. And the noun “evidence” is an uncountable noun.

## Materials and methods

## 1. Annotation in the parentheses:

90% **water dispersible** granules

Comment: Replace “water dispersible” as “water-dispersible”.

2. Where will the scenario of planting *Iris pseudacorus* in such high Cd concentration (above 5.0 mg/L) and simultaneously suffering the atrazine stress appear and what is the significance of this research if this scenario is very special? Please clarify.

3. There are many different leaves on the *Iris pseudacorus*. What’s the rule on selecting the leaves for the chlorophyll fluorescence kinetics measurements?

## 4. Sentence:

Theses parameters derived from the original fast fluorescence transient OJIP measurements were calculated according to Guo et al. (2020).

Comment: Correct “Theses”.

5. How to calculate the parameters of  $\Delta W_L$ ,  $\Delta W_K$ , ABS/RC,  $TR_o/RC$ ,  $DI_o/RC$ ,  $ET_o/RC$ ,  $PL_{abs}$  and  $PL_{total}$ ? Please clarify in Table 1. Also, the JIP-test parameters mentioned in the Discussion section should be briefly introduced in the Materials and methods section. The general readers would be hard to understand what do the JIP-test parameters mean and how to explain the photosynthetic process by these parameters before reading the full text.

## Results

## 1. Sentence:

In addition, the uptrend in  $F_o$  and downtrend in  $F_v/F_o$  with the increase of ATZ concentration were

observed, and the **change** trend showed a significant dose effect relationship (Fig. 1B).

Comment: Replace “change” as “changing”.

2. Sentence:

ATZ alone at 0.1 mg·L<sup>-1</sup> did not differ significantly from the control in  $F_o$ ,  $F_m$  and  $F_v/F_o$ .

Comment: Insert a comma after “ $F_m$ ”. Similar problems exist in the full text, please noted.

3. Sentence:

when ATZ concentrations were 1.0 and 2.0 mg·L<sup>-1</sup>,  $F_o$  and  $F_m$  increased significantly, and  $F_v/F_o$  did not **showed** significant compared with those of the control.

Comment: Corrected the tense of “showed”.

4. Sentence:

ATZ only at levels above 0.1 mg·L<sup>-1</sup> showed significant differences in values of  $V_i$ ,  $V_l$  and  $F_j/F_l$  from ATZ combined with 10 mg·L<sup>-1</sup> Cd, whereas this difference was not observed when Cd concentration was 5 mg·L<sup>-1</sup> (Fig. 2B, D, F).

Comment: Correct the first “ $V_i$ ”. And the significant differences should appear in the 5.0 mg/L Cd treatment, please ascertain the description here.

5. Sentence:

Under single ATZ and combined stresses of ATZ and Cd,  $V_j$  showed **higher** increase magnitude than  $V_l$ .

Comment: Add “a” before “higher”.

6. Sentence:

The initial slope of single **ATZ** at the level of 0.1 and 0.5 mg·L<sup>-1</sup> was significantly greater than that of the control

Comment: Add “treatment” after “ATZ”.

7. Sentence:

In contrast to the control, the plant treated with single Cd showed a significant increase in the initial slope, and there was **significant** difference among **two** Cd concentrations (Fig. 3B, C, E).

Comment: Add “a” before “significant” and “the” before “two”.

8. Sentence:

L-band is known as an indicator of the grouping of the PSII units or energetic connectivity between **antenna** and PSII RCs.

Comment: Add “the” before “antenna”.

9. Sentence:

The double normalized chlorophyll *a* fluorescence curve in the 0.02–2 ms time range revealed the changes of **K-band** (Fig. 5)

Comment: Add “the” before “K-band”.

10. Sentence:

In the JIP-test parameters, the absorbed light energy (ABS/RC), captured light energy (TR0/RC), thermally dissipated light energy (DIO/RC), and the energy used for electron transfer (ET0/RC) on the unit of active

center, were used to indicate the energy transformation in the **RSII** (Liu et al. 2020).

Comment: Is the RSII wrong here? Should it be corrected as “PSII RCs”?

11. Sentence:

For the combined treatment with ATZ and Cd, ABS/RC and DI<sub>0</sub>/RC at **high level** ATZ (1.0 and 2.0 mg·L<sup>-1</sup>) were significantly lower than those of corresponding single ATZ treatments;

Comment: Replace “high level” as high-level”.

12. Sentence:

PI<sub>abs</sub> is the performance index for energy conservation from exciton to the reduction of intersystem electron acceptors, and PI<sub>total</sub> is **performance index** for energy conservation from exciton to the reduction of PSI end acceptors.

Comment: Add “the” before “performance index”.

13. Sentence:

For the treatment of single ATZ or single Cd, PI<sub>abs</sub> (Fig. 7A) and PI<sub>total</sub> (Fig. 7B) declined significantly **compare** with the control.

Comment: Correct the tense of “compare”.

## Discussion

1. I suggested the first paragraph of the Discussion section could be early described in the section of Materials and methods.

2. Sentence:

A gradually significant decline in the ratio  $F_v/F_0$  of treatments with ATZ alone above 0.1 mg·L<sup>-1</sup> displayed that the potential photochemical efficiency of PSII decreased with increasing ATZ concentrations, which was associated with a disruption of water-splitting system (Borkowska 2002). Furthermore, significant changes in  $F_v/F_0$  between ATZ concentrations indicated that  $F_v/F_0$  was a sensitive indicator of ATZ induced limitation of photosynthesis in *I. pseudacorus*.

Comment: The former sentences have stated the change and connotative reason of  $F_v/F_0$ , I suggest delete the repeated statement.

3. Sentence:

A sharp rise of J-step contributed to the large accumulation of  $Q_A^-$ , causing an increase in the net rate of the RCs' closure. As indicated by the larger values of  $M_0$  in treatments of single ATZ relative to the control, also proved that ATZ interrupted PSII electron transport beyond  $Q_A$  (Guo et al. 2020).

Comment: I suggest these sentences should follow the third sentence in this paragraph.

4. Sentence:

This point was proven **matching** very well with the decrease in ET<sub>0</sub>/RC.

Comment: Add the preposition “by” before “matching”.

5. Sentence:

Here, the values of VJ and VI increased, suggesting a decline in the exchange capacity of PQs at the QB site and **reoxidation** capacity of plastoquinol (PQH2).

Comment: Add “the” before “reoxidation”.

6. Sentence:

However, *I. pseudacorus* grown in a medium with ATZ alone of 0.1 showed a negative L-band with significantly decreased WL and FL/FJ, implicating higher connectivity of antennae to RCII units and **more efficient utilization of excitation energy**.

Comment: The inference of “more efficient utilization of excitation energy” seems to be inconsistent with the values of ABS/RC and  $ET_0/RC$ . Please explain.

7. Sentence:

Besides, the overall impact of ATZ on the photosynthesis was likely linked to the oxidative stress in plants trigger by blocking **of** photosynthetic electron transport.

Comment: Replace “of” as “the”.

8. Sentence:

This was consistent with what was indicated clearly by the higher  $ET_0/RC$  and lower  $DI_0/RC$ .

Comment: The  $DI_0/RC$  isn't lower at the combined treatment than the single ATZ treatment below the 0.5 mg/L of ATZ. Please explain.

9. Sentence:

“and a **low** fluorescence intensity at K point relative to ATZ alone, as well as negative L-band values”.

Comment: Delete “a” before “low”.

10. Sentence:

Moreover, the N atoms of ATZ have **stronger** complexation ability with  $Cd^{2+}$  than with other divalent metal ions, such as  $Zn^{2+}$  and  $Cu^{2+}$ .

Comment: Add “a” before “stronger”.

11. Sentence:

A **former** research indicated that at the 1:1 molar concentration ratio, the formation of the complex between ATZ and Cd notably reduced the individual toxicities to rice seedlings.

Comment: Delete “A” before “former”.

12. Sentence:

“for better understanding of the toxicity of **mixture** of ATZ and Cd to plants”.

Comment: Add “the” before “mixture”.

13. Sentence:

“The inference was also supported by the initial slope of the standardized fluorescence transient”.

The alleviating effect of the presence of Cd was ascribed to the formation of the complex between ATZ and  $Cd^{2+}$ . It is a reasonable inference. However, the initial slope of the standardized fluorescence transient also increased at the single Cd treatment comparing with the control. Please explain the phenomenon.

14. Sentence:

For **most of the selected JIP-test parameters**, there were no statistically significant differences between the two Cd concentrations selected in this study.

Comment: Please point out which parameters have no significant change.

15. Sentence:

Therefore, ATZ combined with Cd still had inhibitory effects on **photosynthetic** performance of *I. pseudacorus*.

Comment: Add “the” before “photosynthetic”.

## Conclusion

1. Sentence:

It also had indirect impacts on the donor side because of the imbalance of **electron** in and out of RCs and subsequently the less efficient energy exchange between the neighboring PSII units

Comment: The “electron” should be in plural form.

2. Sentence:

Due to the formation of **ATZ-Cd** complex, ATZ mixed with Cd displayed **weaker** influence on the photosynthesis ability relative to ATZ alone

Comment: Add “the” before “ATZ-Cd” and “a” before “weaker”.