**Reply to the Editor**

Dear Dr. Nedić:

We appreciate the effort, and want to express our thanks to the reviewers for the thorough and extensive review of the paper. We have carefully reviewed the comments and have revised the paper accordingly. We have tried to be thorough in writing this paper and perhaps that’s why we have gone out of the permitted page limit. We also checked and updated language of our paper, as was indicated by Reviewer 1. The changes in the manuscript as well as added text are given in red (Reviewer 1) and blue color (Reviewer 2).

Reviewer 1:

1. *Line 2: excessive instead of exceeded in the title of this paper.*

We have replaced the word exceeded with the word excessive in the title of the revised manuscript.

1. *Line 19: During growing period is not precise, at what stage and how?*

This sentence was extended with the more detailed clarification of the treatments. The plants were treated with heavy metals (Cd and Zn) 24 h after sowing in the open field, at natural conditions. Every plant was watered with 20 mL of 3 g L-1 zinc sulfate anhydrate and 15 mg L-1 cadmium sulfate anhydrate solutions, respectively. The watering of the plants was repeated three times.

1. *Line 27: Does the statement in the last sentence apply to the treatment with or without Zn and Cd presence*

The last sentence of the abstract was modified as follows: Melatonin induced alternation in SOD isoenzyme profiles and activities as well as POD activity in both plant species treated with Zn and Cd.

1. *Lines 111-127: This paragraph is very badly written. The authors should decide if melatonin was used as a treatment, pretreatment or priming? In my opinion the most precise term would be pretreatment and it should be used in the whole text. It is not clear how many times were Melissa seeds sprayed with melatonin, how long was the period between melatonin pretreatment and metal application, how exactly were the plants treated with the metals, had the procedure of submerging seedlings in a solution for 48 h without oxygen ever been applied as melatonin treatment. Besides, authors omitted to describe how and when Valeriana seedlings were treated with the metals.*

Melatonin is applied as *pretreatment* of the plants and this term is used in the revised manuscript. Lemon balm seeds were sprayed daily with melatonin, or water for control, until germination was complete and plants have formed two cotyledons. Just after that time, plants were transferred to a constant place in the open field and within 24 h treated with heavy metals. Same procedure was used for valerian seedlings.

1. *Line 125: after heavy metal and melatonin treatment – these treatments were applied at different times, so it is not clear when exactly the harvest was performed.*

Plant sampling was performed 12 h, 15, 30 and 45 days after heavy metal treatment, as corrected in the reviewed paper. Melatonin was used as a pretreatment.

1. *Line 222: Why should not suppression of melatonin biosynthesis by exogenous melatonin be considered? If weather conditions are to be accused, it should be specified what they were at the time of sampling.*

Lemon balm samples were harvested in summer time, at higher temperatures, and valerian in the fall, when the temperatures are not so devastating. Numerous papers state the effect of sunlight, among others, on the melatonin level in plants.29 We interpret our results as a possible effect of sunlight on the steady-state of melatonin utilization. Due to melatonin consumption in its role as antioxidant it is likely that the higher the sunlight the lower the melatonin level. This statement is in accordance with previous research in temperature effect on melatonin level in plants.30 Also, the decrease of melatonin in plant leaves may be a response of plants to re-direct this compound to another plant organ, what will be the subject of future research. In our opinion more data are necessary to understand the physiological role of melatonin in plants under natural conditions.

1. *Lines 230-246: This paragraph should be incorporated in Introduction and the language should be checked once more.*

We have incorporated this paragraph into Introduction.

1. *Line 248: Does this imply soluble proteins?*

Yes, it means soluble proteins. The content of soluble proteins in lemon balm and valerian leaves is given in Table II of revised manuscript.

1. *Line 256: The developmental stage should be specified.*

The development stage is now indicated and the protein content described in the following sentence:

The most prominent increase of soluble protein content was observed in the samples of lemon balms after 30 days regardless of the treatment. Particular increase was detected in the samples treated with Zn and M+Cd, at the same developmental stage, 30 days after heavy metal treatment.

1. *Line 264: This is not true. The results show that the treatments with the smallest concentration of endogenous melatonin are not those with the most reduced protein concentration at the final development stage.*

We have changed our statement as follows: Our results indicate that smaller concentration of melatonin may lead to reduction of soluble protein concentration at the final development stages in lemon balm and younger valerian leaves when treated with heavy metals and pretreated with melatonin.

1. *Table I. Statistical significance of the results is lacking*

Statistical data for the soluble protein content are added in Table I, which became Table II in reviewed paper.

1. *Lines 279-293: Nothing is mentioned about IbSOD2 band*.

We added a description for lbSOD2 band. lbSOD2 isoform is present in all developmental stages of the plant and within all treatments.

1. *Figures 6 and 7. The figures are too small, the columns are indistinguishable, there are not letters above each column.*

The figures are redone by reviewer’s suggestions in terms of distinguishing columns and adding all the letters above the columns.

1. *Lines 377-391: What about comparison between M+Cd or M+Zn with Cd and Zn applied alone?*

Since the comparison between M+Cd or M+Zn with Cd and Zn applied alone was omitted we added paragraph as follows: There is no significant difference in POD activity between M+Cd or M+Zn compared to Cd and Zn alone, except in M+Cd treatment at first developmental stage. Interestingly, POD activity was enhanced in M+Cd and especially M+Zn compared to Cd and Zn alone at first developmental stage of valerian, but same or decreased in all other stages.

1. *Lines 393-400. This part should be incorporated into Introduction.*

That part has been moved and incorporated into Introduction.

1. *General remark: The point of this study – to investigate the influence of exogenous melatonin on plant response to Zn and Cd appears to be lost and it is nowhere described explicitly. Besides, some appropriate statistical tool, like two-way ANOVA should be used to measure the contribution of metals on one hand, and of exogenous melatonin treatment on the other.*

In order to further explain the influence of melatonin in plants we briefly address this issue in the conclusion section.

Results of this paper support the theory that melatonin might protect the plants from stress conditions and prevent injuries induced by oxidative stress at the cellular level especially in valerian, by elevating the activity of antioxidative enzymes in plant first developmental phases. Such a response could be explained in two ways: first, melatonin is a receptor-independent free-radical scavenger and a broad-spectrum antioxidant, second, it can stimulate antioxidant enzymes or augment the activities of other antioxidants to protect plant tissues from oxidative damage.

Also, two-way ANOVA was conducted, and the contribution of melatonin pretreatment and heavy metal treatments was measured.

1. *Line 231: It would be interesting to specify the kind of stress*

In reviewed paper we have specified the kind of stress was used in cited paper. Namely, it was showed that treatment with 1 mM zinc sulphate in 9-day-old barley plants increases endogenous melatonin content for a six fold in barley root.30

1. *Lines 328-331. In my opinion this is not true. Protein accumulation is actually the reflection of antioxidant enzyme, signal compound and transcriptional factor accumulation.*

This part of the text has been deleted.

1. *The language should be checked once more.*

Reviewer 2:

1. *It is important to inform how the proteins were extracted. Please, give more details about extraction procedure*

In order to better clarify the extraction procedure of the proteins we have modified this section in materials and methods accordingly. Same extraction procedure of proteins and enzymes was conducted, and now it is explained better in reviewed paper. The leaf tissues (0.5 g) were powdered in liquid nitrogen, homogenized in 4 mL of 100mM sodium phosphate buffer (pH 6.4) containing 1mM PMSF, 0.2% TWEEN and 2% PVP. The extract was centrifuged at 4 °C for 10 min at 10.000 rpm and the supernatant containing soluble proteins was used to determine protein concentration and enzyme activity.

1. *The melatonin levels are expressed as μg per g. For completely clarity the author should explain the origin of g (What does mean g- gram fresh weight?).*

In reviewed paper we clarified the unit of obtained melatonin concentration. The final concentration is expressed as mg/g dry weight

1. *There are no error bars in Table I and there is no indication that replicate experiments were carried out*.

Considering the lack of statistical significance of the protein concentration results, we have modified the table in reviewed paper.

1. *The authors should present the ratio of fresh weight to dry weight (FW/DW) of plant leaves, an index of cell water content. The water content in the tissue can change, which can be expected for leaves from July and October.*

Unfortunately, the water content in plants has not been determined. Since the enzyme (POD and SOD) activity was expressed as a specific activity relative to the protein content, and the content of the metal and melatonin expressed on the leaves dry weight, the influence of water is somewhat avoided. Future research will certainly include this important parameter.

We hope that you find our comments and revisions acceptable.

Sincerely,

dr Biljana Kukavica

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