Calcium defeating strategy for metal stress in some submerged hydrophytes growing in aquaponics

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Research Article

Keywords: Calcium, Cell signaling, Metal stress, Hydrophyts, Hydroponics

Posted Date: April 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1519114/v1

Abstract

The present work is a fetching trial providing promising insights about calcium. Calcium plays a crucial role in plant nutrition; moreover Calcium ions (Ca^{2+}) show a versatile propensity in plant structure and function. Introducing heavy metals especially Cadmium to plant interrupts many biotic processes which ultimately lead to plant death. Herein, from submerged hydrophytes, *Ceratophyllum demersum*, *Myriophyllum spicatum* and *Najas marina* are examined to evaluate their tolerance against metal stress. All of them are subjected to a mixture containing different doses of cadmium with fixed doses of lead and nickel in hydroponic culture for one week. All of inspected species exhibit analogous response regarding calcium elevation level. The observed positive correlation between Cd and Ca is attributed to invigorating role of calcium in plant resistance. Calcium is incorporated in plant defense strategy to both environmental and hormonal signals. The involvement of calcium in reducing metal uptake particularly Cd influx and translocation, controlling signal transduction and alleviating oxidative damages in plant explains the reasons beyond the transit cytosolic calcium increase in all selected species.

Introduction:

Heavy metals (HMs) show ubiquitous source of plant stress (Al-Whaibi 2011). They are naturally occurring and increases with anthropogenic activities (Spiegel 2002). Discrimination of these kind of metals depends on their specific gravity which exceeds that of water by five folds and have an atomic number more than 20 (Ali et al. 2019). Ni, Cd and Pb draw attention as powerful pollutants (Amari et al. 2017). Due to their persistence, mobility and un-biodegradability, HMs spreads reaching aquatic systems (Wieczorek-Dabrowska et al. 2013). By time aquatic ecosystems quality is declined by piling of pollutants (Ansari et al. 2020).

Most of Submerged species have a privilege of being anchored thus they are vulnerable enough to reflect environmental conditions impacting plant growth rather than moving biota (Zhou et al. 2017).

Heavy metals display the ability to produce reactive oxygen species (ROS), causing lipid peroxidation, DNA damage and altered calcium homeostasis (Martínez Domínguez et al. 2009; Redza-Dutordoir and Averill-Bates 2016). Owing to different stress conditions, Plants pass through a variety of changes in a quest to mitigate such adversities caused by abiotic stresses (Hussain et al. 2018). Calcium ions (Ca^{2+}) elevation serve as an important plant response while facing environmental cues as it has been related to signaling network of plant (McAinsh and Pittman 2009; Dodd et al. 2010). Also, precipitation of Ca-oxalate in plants, including floating macrophytes have been reported in metal toxicity counteraction (Jáuregui-Zùñiga et al. 2005).

Calcium is one of the most frequent metals on earth's crust and it is one of essential macroelements in plant nutrition (Himschoot et al. 2017). As a divalent cation, it acts dually in both levels of plant structure and development as well as plant functions (Sanders et al. 2002; Tuteja 2007). It is essential in middle lamella of recently synthesized cell wall (Sanders et al. 1999). In plant membranes, calcium stabilizes lipid bilayers and also implicated for normal functioning of plant membranes via its incorporation to phospholipids. and thus provides structural integrity to cellular membranes (McAinsh and Pittman 2009).

Unlike animals, plants have not the capability to run away from external stimulus; therefore cells are programed with intricate network of messengers to transmit signals into the intracellular response. As a second messenger, Calcium is one of the crucial signaling agent (Jalmi et al. 2018). ROS trigger releasing of cytosolic free Ca^{+2} which in turn activate Ca^{+2} Channels (Choi et al. 2017). Myriads of calcium sensing proteins like Calmodulins (CaMs), CaM like proteins (CMLs), Calcineurin B-like proteins (CBLs), and Ca^{+2} -dependent protein kinases (CDPKs) which bind to Ca^{+2} can recognize this transient increase in the cytosolic concentration. These calcium sensing proteins contribute in transduction of chemical signal into a biological response (Steinhorst and Kudla 2014).

Many studies depicting some fluctuations of Ca^{+2} level in plant as an adaptive mechanism to alleviate toxic effects of heavy metal. Plants exposed to cadmium exhibit a higher level of intracellular Ca^{+2} (Yang and Poovaiah 2003). In response to toxicity mediated by heavy metals like lead (Pb^{+2})and nickel (Ni^{+2}), Ca^{+2}/Calmodulin system is also involved (Ahmad et al. 2015).

It is well known that water plants are exposed to various abiotic stresses in their natural habitats. In the current work, it will be highly advantageous to outline the relation between stress caused by a mixture of three heavy metals (Cd, Ni and Pb) and calcium level in three submerged hydrophytic species. *Ceratophyllum demersum, Myriophyllum spicatum* and *Najas marina* are sensitive to the ambient circumstances thus they are good paradigm in this approach.

Materials And Methods:

Plant Material:

Ceratophyllum demersum, Myriophyllum spicatum and *Najas marina* were collected from their natural watercourses in Fayoum depression-Egypt.

Metal Treatment Experiment:

All chemicals and reagents used in this study were purchased in analytical purity grade from Sigma Aldrich (Munich, Germany), VWR (Darmstadt, Germany).

Apical parts of almost same age with uniform size and surface area were cut out from hygienic plants individuals. Cut segments were rinsed thoroughly and distributed in sets of aquaria filled with 0.1% Hoagland's nutrient solution (Hogland 1950). From previously prepared metals stock solutions, aquaculture medium was augmented with the following concentrations according to preliminary test:

1) For *Ceratophyllum demersum*, L

Plants were exposed to equal concentrations of Pb and Ni (5 mg L^{-1}**)** and serial concentrations of Cd (0.0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mg L^{-1}).

2) For *Myriophyllum spicatum*, L.

Plants were exposed to equal concentrations of Pb and Ni (50 mg L^{-1}) and serial concentrations of Cd (0.0, 10, 20, 30, 40and 50 mg L^{-1}).

3) For *Najas marina*, L.

Plants were exposed to equal concentrations of Pb and Ni (30 mg L^{-1} and 10 mg L^{-1} respectively) and serial concentrations of Cd (0.0, 50, 100, 150, 175and 200 mg L^{-1}).

The experiment was lasting for one week at constant temperature of 25 ± 2°C. Photoperiod was set up as 16 h light/ 8 h dark for a daily cycle. Plants were harvested after first day, fourth day and seventh day of exposure, washed with demineralized water, blotted and subjected to calcium determination.

Determination Of Calcium Content:

Plant materials were digested with nitric acid and hydrogen peroxide. Using inductively coupled plasma mass spectroscopy -Agilent 7500a, USA (ICP), the digest material was analyzed for Calcium.

Statistical analysis:

All analysis and plots were done using the R programming language (version 4.0.2.) For each plant, the linear mixed model was built for predicting Calcium by using day, cadmium, and nickel as fixed effects, cadmium as a random slope, and day as a random intercept using the lmer function from the lmerTest package (Kuznetsova A et al. 2017) of R programming language (R Core Team 2020). Then, we used the step function, from the same package, which did a backward elimination to determine the important fixed and random effect terms.

To plot the model predictions along with the raw data, we used different functions from the tidyverse (Wickham et al. 2019), broom (David Robinson et al. 2020), broom.mixed (Ben Bolker and David Robinson 2020), and sjPlot packages (Lüdecke D 2020) of R.

Differences among means were calculated using the LSD (least significant difference) range test with a family error rate of 0.05. LSD was performed using Statistica software (Weiß 2007).

Results:

The results of inductively coupled plasma mass spectroscopy (ICP) clarified the progressive Ca increase in correspondence with successive Cd addition to the medium in all tested plant species. Intuitively the elevation of Ca content with increasing Cd concentration was accompanied with constancy of Pb and Ni supplementation.

Statistica software employment implied significant differences among calcium magnitudes in response to cadmium which is obvious in tables S1, S2 and S3.

As the same plant was measured thrice for a week (at days 1, 4, 7), a linear mixed effect model was thought to predict the Calcium concentration using the day of exposure, cadmium, nickel, and lead. Because nickel and lead concentrations were perfectly collinear, this produced singularities in the regression model, and only nickel was included.

Initial exploratory analysis for the relation between calcium and all these variables had shown that data nearly fit a random slope by cadmium and a random intercept by the day of exposure. The large values of calcium were scaled by log transformation with base 10 and the day of exposure was scaled to begin at zero time instead of one.

All linear mixed effect models showed improved R squared value and residuals appearance over their linear model counterparts. The R squared was calculated using the rsquare function from the modelr package (Hadley Wickham 2020) of R, while plotting the residuals was done using the plot function from the base package (R Core Team 2020) of R.

Figures (1, 2 and 3) evinced a noticeable same behavior of *C. demersum*, *M. spicatum* and *N. marina* regarding Calcium level with elapsed time. *M. spicatum* showed 5.6 % increase in Ca for each one day of exposure where R squared increased to 91.5 %.

Discussion:

Calcium ions is a part of signaling network of plant reactions against both biotic and abiotic stress (Dalcorso et al. 2010). It is obviously known that calcium as a universal second messenger and its conjugated proteins can mitigate hazards originates from metal stress. Also, calcium ions minimize negative charge of cell surface keeping cationic toxicants away (Yang and Poovaiah 2003). In addition, its binding to phospholipids guarantee the structural integrity of plasma membrane by stabilizing lipid bilayer which is subjected to severe deterioration provoked by heavy metals (Gonnelli et al. 2001; Hepler 2005).

The current study revealed the directly proportional correlation between cadmium and calcium at constant concentration of both lead and nickel. Significant increase of calcium may invigorate all tested plant species against cadmium stress.

As a result of Cd, Ni or Pb gripping, cell wall plasticity and microtubules network are adversely affected. This disruption imperiled cell division and activities (Wierzbicka 1998).

On one hand, Cd, Ni and Pb entry to the cell was through Ca transporters. On the other hand a strong competition appeared between calcium and some other heavy metals (Rodríguez-Serrano et al. 2009). Cd and Ca ions are physio-chemically alike. The resemblance of Ni^{+2} and Cd^{+2} ion radii to that of Ca^{+2} enriched the potentiality of their uptake via Ca^{+2} gated channels (Choong et al. 2014). Cd, Ni and Pb uptake through Ca transporters is in agreement with (Markich et al. 2006).

In cytosol, Cd and Ni as well as Pb are destructively denaturing total protein content in many plant species (Mishra et al. 2006). Not only protein quantitative changes were recognized but also enzymes activities of various metabolic processes are aggravatingly influenced by Cd, Ni or Pb (Taamalli et al. 2015).

Many authors emphasized this destructive role of heavy metals. (Baccouch et al. 2001) demonstrated similar results in nickel treated maize where (Liu et al. 2011) found the same approach in *Suaeda salsa*. (Małkowski et al. 2005) verified the positive relationship between lead and calcium in corn seedling roots.

The major target of calcium signals are calcium-modulated proteins (Yang and Poovaiah 2003). Moreover, elevation of cytosolic Ca^{+2} level enhance its combination to calmodulin protein which in turn controls a lot of physiological and biochemical responses inside the cell. In chloroplast, calcium converts NAD^{+} to NADP which is the final acceptor of electrons. On the contrary, cations of HMs adverse the process of electron transport by declining cytosolic free calcium during photosynthesis leading to ROS formation (Cruz De Carvalho 2008). (He et al. 2005) found that Ca^{+2} triggers phytochelatin synthase gene expression in order to improve plant tolerance against cadmium stress. Furthermore, in Arabidopsis seedlings, (Zhao et al. 2014) noticed the role of Ca^{+2} in auxin homeostasis in facing of Cd effects. (Kania et al. 2017) reported that inositol1, 4, 5-triphosphate (IP3) production stimulates discharge of sequestered calcium from its inventory.

(Małkowski et al. 2005) proved that cadmium-calcium correlation is more coherent through plasma membrane in maize seedlings compared to lead-calcium one which is stronger at apoplast level in maize seedlings.

Additionally, exogenous application of calcium enabled plant to diminish cadmium uptake and to quench its toxicity. This relation was reported by (Ahmad et al. 2015) in a study of *Brassica juncea* and by (Wan et al. 2011; Tian et al. 2011 ; Farzadfar et al. 2012) in some other plants like *Brassica napus, Matricaria chamomilla and Sedum alfredii*.

Lastly, the selected plant species were recorded for their accumulation ability of metal, the privilege that allowd these species to withstand in front of their enemies of metals. *C. demersum* has been demonstrated as good accumulator for Pb and Cd. *M. spicatum* and *Najas indica* were reported for Cd and Pb respectively (Singh et al. 2010; Ciszewski et al. 2013; Andresen et al. 2016).

To conclude, the ongoing work tries to describe the great battle among Calcium and HMs especially cadmium in which calcium still struggling till plant life termination. Herein, the more exposure to HMs, the more calcium efflux appears. So, calcium still increases while coping with high concentrations of metal.

Conclusion:

The presented data is obviously explained calcium role in amelioration of plant cells while confronting various doses of HMs. When the medium is supplemented with ascending cadmium concentration along with lead and nickel, growing plant species suffer from ROS and its broad spectrum of hazards. Calcium as a trafficking agent can almost combat these hazards. Significant rising of calcium level throughout the experiment is a strong indication of its protection ability to tested plants. Production of calcium signal followed by signal decoding in *C. demersum, M. spicatum* and *N. marina* is the key process to overcome the temporary changes caused by metal stress.

Statements & Declarations:

The author declares that no funds, grants, or other support were received during the preparation of this manuscript.

Data Availability Statement:

The data presented in this study are available in this article and the Supplementary Materials.

Conflict of interest:

There is no conflict of interest as the current paper is single authored one.

Funding:

This research received no external funding.

Competing Interests

“The authors have no relevant financial or non-financial interests to disclose.”

Author Contributions

Single author

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54. *The author declares that no funds, grants, or other support were received during the preparation of this manuscript*.
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Figures

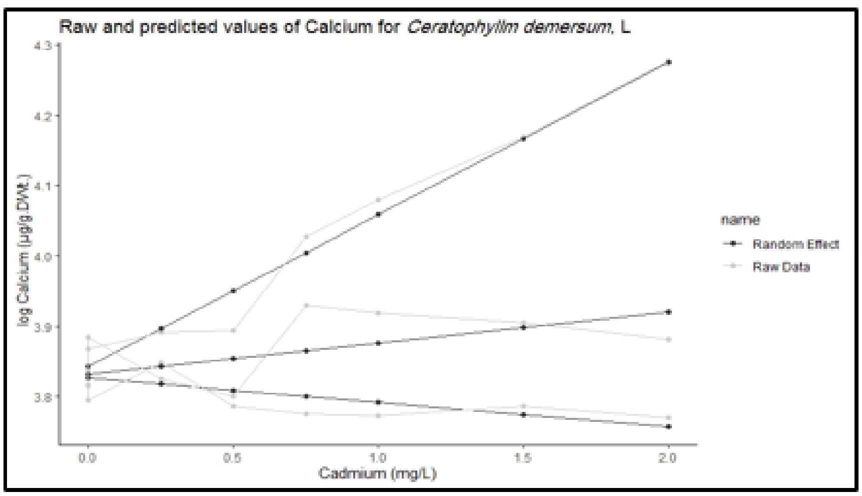

Figure 1

Effect of various Cd concentrations combined with 5 mg L^{-1} of Pb and Ni on Calcium level in *C. demersum* after 1, 4 and 7 days of exposure.

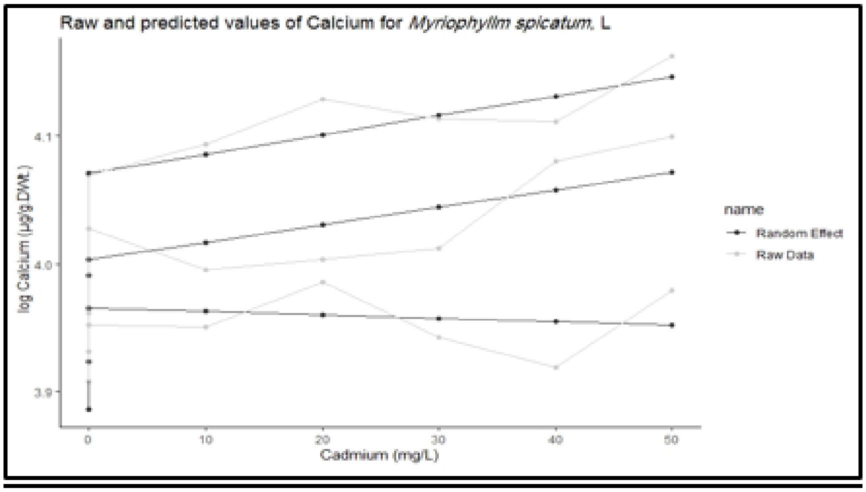

Figure 2

Effect of various Cd concentrations combined with 50 mg L^{-1} of Pb and Ni on Calcium level in *M. spicatum* after 1, 4 and 7 days of exposure.

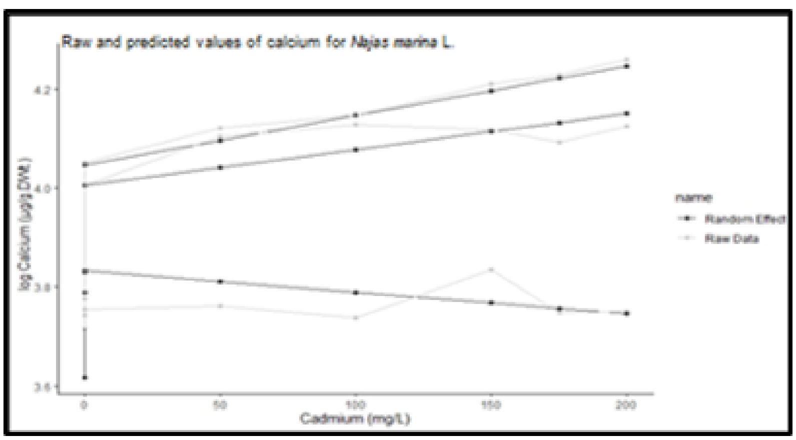

Figure 3

Effect of various Cd concentrations combined with 30 mg L^{-1} of Pb and 10 mg L^{-1} of Ni on Calcium level in *N. marina* after 1, 4 and 7 days of exposure

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx