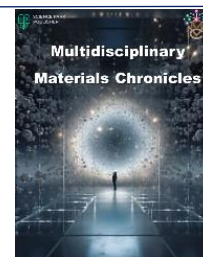


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Multidisciplinary Materials Chronicles

Comparative zinc uptake and metabolic responses of *Ipomoea aquatica* to hydroponic exposure of zinc sulphate, zinc oxide, and zinc oxide nanoparticles

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Abstract

This research aimed to investigate zinc (Zn) accumulation patterns and the resulting metabolite-profile alterations in *Ipomoea aquatica* (*I. aquatica*) (water spinach) when hydroponically exposed to 50.0 mg/L concentrations of zinc sulphate (ZnSO₄), zinc oxide (ZnO), and zinc oxide nanoparticles (ZnO NPs). Roots consistently exhibited the highest Zn accumulation across all treatments. ZnSO₄ exposure led to the maximum concentration of 4620 ± 458 mg/kg DW in roots, while ZnO NPs resulted in 2830 ± 321 mg/kg DW and ZnO in 957 ± 40.5 mg/kg DW in roots. Stems generally showed the lowest Zn accumulation, with the control at 43.2 mg/kg DW, though ZnSO₄-treated stems had the highest accumulation among treated groups at 962 ± 19.2 mg/kg DW. The overall Zn uptake in all biological parts (leaves, stems, and roots) followed the order: ZnSO₄ > ZnO NPs > ZnO > Control. GC-MS analysis revealed that all Zn treatments significantly altered *I. aquatica*'s metabolite profile, resulting in a drastically reduced diversity of detectable metabolites compared to the control. For instance, the number of consistently detected metabolites plummeted from 45 in the control to just 6 in ZnSO₄, 9 in ZnO, and 7 in ZnO NPs treatments. Moreover, the mean peak height, an indicator of overall metabolite abundance, sharply declined from 486000 ± 36000 in the control to 4600 ± 462 in ZnO NPs exposure. While cyclooctasiloxane (hexadecamethyl) and cyclononasiloxane (octadecamethyl) were consistently detected in all treatments, cyclohexasiloxane (dodecamethyl) and cyclopentasiloxane (decamethyl) were exclusive to Zn-enriched experimental sets, and cyclotetrasiloxane (octamethyl) was specifically associated with ZnO and ZnO NPs exposure. Notably, silicic acid (diethyl bis(trimethylsilyl) ester) and pyridine-3-carboxamide (1,2-dihydro-4,6-dime) were uniquely detected in the leaves of plants exposed to ZnO NPs. This suggests potential roles in toxicity mediation, possibly through a silica coating effect reducing Zn²⁺ dissolution, or altered metabolic pathways, given pyridine-3-carboxamide's diverse biological activities, which include antiviral, antibacterial, and anticancer properties. These findings collectively underscore how the form of Zn critically influences its bioavailability, accumulation patterns, phytotoxicity, and distinct metabolite profile alterations in *I. aquatica*, providing crucial insights for optimizing agricultural practices and environmental management strategies, particularly regarding phytoremediation in heavy metal-contaminated environments and micronutrient management.

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Keywords: Zinc, zinc oxide nanoparticles, hydroponic, *Ipomoea aquatica*, metabolite profiling

1. Introduction

Zinc oxide nanoparticles (ZnO NPs) are widely used in biomedical and industrial applications due to their unique properties [1]. Given their widespread applications, ecotoxicological research on ZnO NPs is essential to assess their environmental impact. Environmental concentrations of ZnO nanoparticles (NPs) vary across different compartments: surface waters range from 0.001–0.058 g/L, soil from 0.24–0.661 g/kg, and wastewater treatment plant effluent from 0.22–1.42 g/L [2]. Sludge used as fertiliser can contain up to 3000 mg/kg of ZnO NPs [3]. Specific studies have measured concentrations such as 4.4×10^5 particles/mL in Canadian rivers [4] and 20.0–212.0 µg/L in Maryland wastewater [5]. With the increasing use and discharge of ZnO NPs, these concentrations are expected to rise. However, data for Malaysia's ecosystem remains scarce, emphasizing the urgent need for further research on their ecological impacts.

Ipomoea aquatica (water spinach) ability to thrive in wastewater environments is a highlight of its potential in mitigating heavy metal pollution while acting as a food source for human populations [6, 7]. Its ability to bioaccumulate metals like Zn makes it a useful model for ecotoxicity assessment and phytoremediation studies [8-10]. Investigating its response to different Zn sources, ZnSO₄, ZnO, and ZnO NPs, can provide insights into metal uptake, toxicity mechanisms, and metabolic adaptations.

Metabolite profiling is a powerful tool in heavy metal ecotoxicology, revealing metabolic disruptions caused by metal exposure and aiding in biomarker identification [11]. Heavy metals can alter key metabolic pathways, particularly amino acid and lipid metabolism, triggering oxidative stress responses [12]. Integrating metabolite profiling with ecotoxicological investigations offers a powerful approach to elucidate plant adaptation and detoxification mechanisms in response to heavy metal stress. The study of metabolite profile changes in plants exposed to various Zn forms, such as Zn²⁺ and ZnO NPs, is essential for understanding their physiological responses and overall health. Metabolite profiling highlights how plants adapt to Zn sources by revealing alterations in core and secondary metabolic

pathways. ZnO NPs significantly affect the plant metabolome, modifying levels of amino acids, organic acids, carbohydrates, and secondary metabolites like flavonoids, which are vital for growth and stress tolerance [13]. These shifts suggest ZnO NPs not only boost beneficial compound accumulation but also enhance antioxidant defence pathways, improving plant resilience to environmental stress [14]. Additionally, ZnO NPs can promote growth by increasing biomass and nutrient uptake, though higher concentrations may impair these processes due to potential toxicity [15]. Their ability to alter gene expression linked to metabolite synthesis further positions ZnO NPs as effective nanofertilisers [16].

The objectives of this study are to study the Zn accumulation patterns on water spinach (*I. aquatica*) hydroponically exposed to 50 mg/L of ZnSO₄, ZnO, and ZnO NPs and the resulting differences in metabolite profile of *I. aquatica* in response to these treatments.

2. Materials and methods

2.1. Zn pollutants and their properties

This exposure experiment involved zinc sulphate heptahydrate (ZnSO₄·7H₂O), zinc oxide (ZnO), and zinc oxide nanoparticles (ZnO NPs). They were sourced from Bendosen Laboratory Chemicals, Malaysia, Aladdin Bio-Chem Technology Co., Ltd. (product ID: Z141332), and Sigma-Aldrich, USA (Product ID: 721077), respectively. All the chemicals involved in this research were of analytical grade and used without further purification.

The spherical shape of ZnO NPs, with a diameter of approximately 44 nm, was revealed by TEM analysis. It was shown by Dynamic Light Scattering (DLS) analysis that the average hydrodynamic diameter of ZnO NPs changed to about 198 nm with a zeta potential of -22.2 ± 0.13 mV. This hydrodynamic diameter, being almost three times larger than the size discovered in TEM, indicates that ZnO NPs generally tend to agglomerate in various media during exposure to biological systems [17].

2.2. Experimental design and exposure

The experiment was conducted from 24 June to 22 July 2024 in a greenhouse at the Department of Biology, Faculty of

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Science, University Putra Malaysia. Germination took place in non-circulating polymer hydroponic tanks with 16 litres of tap water, prepared a week in advance. To ensure redundancy, four water spinach seeds were sown per hole and germinated for seven days. Three uniform seedlings per hole were then selected for exposure, with Zn contaminants introduced simultaneously. Hydroponic fertilisers followed Gibeau et al. [18], adapting Hoagland's solution [19] with 1/3-strength macronutrients to minimise osmotic effects and full-strength micronutrients to prevent depletion. Fe-EDTA was prepared as recommended by Steiner & van Winden [20].

Upon harvesting, *I. aquatica* samples were immediately separated into their constituent parts: leaves, stems, and roots, after which their heights were measured. Each biological component was subsequently finely chopped to ensure uniformity. Following this, the dissected samples were oven-dried at 60 °C until a consistent dry weight was achieved [21-23].

2.3. Acid digestion and metal analysis

To quantify Zn concentrations, a 0.5 g sample of *I. aquatica* underwent acid digestion. This involved initial heating at 40 °C for one hour, followed by a prolonged period of at least three hours at 140 °C in a tube containing 10 mL of nitric acid [24]. The resulting digestate was then diluted to a final volume of 40 mL with ultrapure water and filtered using Whatman No. 1 filter paper to remove particulate matter [21, 24]. The filtered digestates were stored in sterile polypropylene containers at room temperature until analysis. Zn concentrations were determined using an air-acetylene flame atomic absorption spectrometer (FAAS, Thermo Scientific iCE 3000, Thermo Fisher Scientific, USA). Instrument precision was ensured through calibration with a series of standards and resetting to zero with ultrapure water before each analytical session.

2.4. Leaf metabolite extraction and metabolite profiling by GC-MS

Leaf samples were collected after a 28-day hydroponic growth period, specifically 1.0 g fresh weight of second leaves (located below the apical leaves), harvested at midday. The harvested leaf tissue was immediately frozen in liquid nitrogen and pulverised using a mortar and pestle.

Metabolites were extracted using a cold solvent mixture of chloroform, methanol, and ultrapure water (100:235:40 µL). Homogenised samples were subjected to vortexing, incubation on ice for 30 minutes, and centrifugation at 1400 rpm for 5 minutes at 2 °C. The supernatant was collected, and the remaining pellet was re-extracted with an additional 300 µL of the extraction solvent. The combined supernatants were then stored at -80 °C prior to Gas Chromatography-Mass Spectrometry (GC-MS) analysis [25].

GC-MS analysis was conducted using a Shimadzu GCMS-QP2010 Plus system equipped with a ZB-5MS capillary column. A 1 µL sample volume was injected in split mode (5:1), with helium acting as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature programme commenced at 50 °C (held for 3 min), increasing to 330 °C (held for 10 min). Both the injector and detector temperatures were maintained at 320 °C and 330 °C, respectively. Mass spectrometry parameters included an ionisation potential of -0.40 kV, ion source temperature 240 °C, a solvent delay of 2.50 min, and a scan range of 35–500 amu. Compound identification within the crude extracts was achieved by comparing mass spectra fragmentation patterns [26, 27] and through computer matching with the Wiley 229 and National Institute of Standards and Technology (NIST) 08 library data.

2.5. Quality assurance

To minimise contamination, all equipment and glassware were pre-treated by immersion in a 5% nitric acid solution for 72 hours, followed by thorough triple rinsing with distilled water and a double rinse with ultrapure water. The accuracy of measurements was validated by processing, digesting, and analysing Certified Reference Materials (CRMs) concurrently with the samples. Specifically, CRM GBW10048 celery (representing plant material) and MESS-3 Marine Sediment (representing soil) were used, yielding Zn recovery rates of 108.95% and 81.63%, respectively.

2.6. Statistical analysis

Statistical analyses, including correlation coefficients and hierarchical cluster analysis, were performed using SPSS Statistics version 21 (IBM Corp.). Single linkage Euclidean distance clustering was specifically employed to evaluate metal accumulation patterns across sampling sites and within different biological components of *I. aquatica*. The statistical

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significance of observed variations was determined using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls (SNK) post hoc test [28]. Although raw data exhibited normal distribution, $\text{Log}_{10}(\text{mean}+1)$ transformed data were used for correlation and cluster analyses. This transformation was chosen due to its empirically observed enhancement of analytical outcomes compared to untransformed data [29].

3. Results and discussions

3.1. Accumulation of Zn in the biomass of *I. aquatica*

Figure 1 details the Zn concentration (mean $\pm$ standard error) in leaf, stem, root, and yellowed leaf (where applicable) of *I. aquatica* exposed to 50 mg/L of various Zn forms, ZnSO_4 , ZnO, and ZnO NPs. The results show a clear increase in Zn accumulation with all applied zinc forms compared to the Control group. ZnSO_4 consistently demonstrated the highest efficacy in increasing Zn content across all tested plant parts, including the Leaf (562 ± 0.180), Senescence/Yellowed tissue (197 ± 0.997), Stem (962 ± 19.2), and particularly the Root, which recorded an exceptionally high Zn content of 4620 ± 458 . Roots exhibited the highest levels of Zn accumulation across all zinc treatments, indicating they are a primary site of

uptake and storage. ZnO NPs generally resulted in higher Zn accumulation (2830 ± 321 in Root, 305 ± 3.18 in Leaf) than bulk ZnO (957 ± 40.5 in Root, 188 ± 0.283 in Leaf) across the respective plant parts, suggesting a more efficient uptake or bioavailability of the nano-form.

Observing the data patterns, the roots consistently exhibit the highest Zn accumulation across all treatments. This is a common physiological response in plants, as roots are the primary site of nutrient uptake from the growth medium. Specifically, for ZnSO_4 exposure, the root accumulated an astonishing 4623 mg/kg DW Zn, marking the highest concentration among all treatments and plant parts. In contrast, the lowest Zn accumulation generally occurred in the stem, with the control group's stem having a particularly low mean of 43.2 mg/kg DW. Among the treated plants, the stem of the ZnSO_4 -treated group showed the highest stem accumulation (963 mg/kg DW), while the stem of the ZnO-treated group had a comparatively lower accumulation (129 mg/kg DW).

For dry weight-based (DW) Zn concentration (mg/kg DW, Table 1), the current study found Zn concentrations of (52.5 - 562) mg/kg for Leaf, (125 - 198) mg/kg for Stem, (615 - 760) mg/kg for Shoot, and (102 - 5080) mg/kg for Root. These values can be compared with previously reported DW concentrations from other locations.

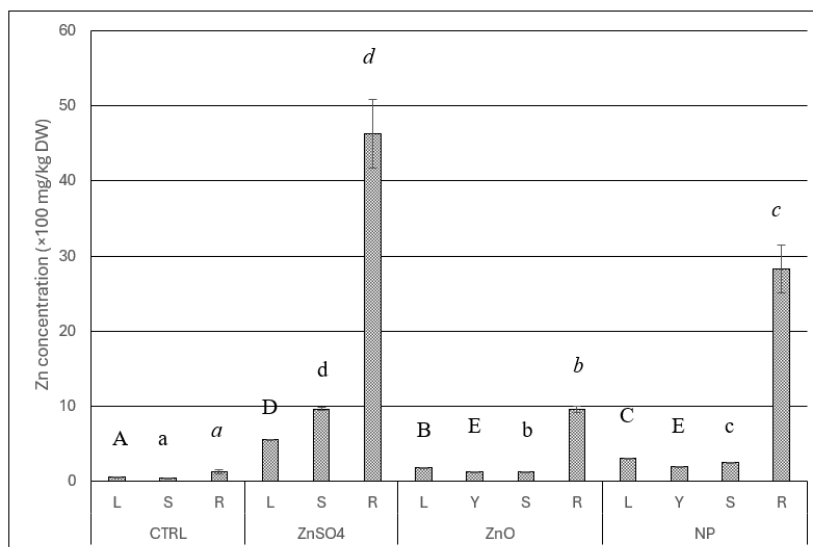


Figure 1. Zn accumulation ($\times 100$ mg/kg DW) in different tissues of *I. aquatica*. Tissues include leaf (L), chlorotic/yellowish leaf (Y), stem (S), and root (R), following exposure to 50 mg/L Zn via ZnSO_4 , ZnO, and ZnO NPs.

Note: Different letters above each column indicate statistically significant differences SNK test; $p < 0.05$) among the respective tissue types across all treatments.

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For instance, a previous study in Seremban, Malaysia, reported 125 mg/kg for Leaf and 103 mg/kg for Stem [30], which fall within or below the ranges of the current findings. In comparison to Nal Sarovar Bird Sanctuary, India, which reported 639.04 mg/kg, the current Shoot concentration of (615 - 760) mg/kg [31] is comparable, and the Root concentration can be significantly higher. However, the current DW levels for Leaf, Stem, Shoot, and Root are notably higher than those reported for Hanoi, Vietnam at 51.5 mg/kg [7] and significantly exceed the 7.055 mg/kg found in Hunan, China [32]. When compared to Malackochha, Sherpur, Bangladesh at (157.63 - 298.64) mg/kg, the current Stem range partially overlaps, while Leaf and Root ranges are much broader, and Shoot is higher [33]. Furthermore, the current Root (102 - 5080) mg/kg and Shoot (615 - 760) mg/kg concentrations are substantially higher than those reported for University of Malaya, Malaysia, which were (1.71 - 34.70)

mg/kg for Root and (1.42 - 35.10) mg/kg for Shoot [34].

Regarding wet weight-based (WW) Zn concentrations (mg/kg WW, Table 1), the current study found (5.77 - 61.8) mg/kg for Leaf, (13.8 - 21.8) mg/kg for Stem, and (4.70 - 108) mg/kg for Shoot. When compared to previous WW findings, a 2021 study from Seremban, Malaysia reported 13.4 mg/kg for Leaf and 10.3 mg/kg for Stem [30], which are within or below the currently observed ranges for these tissues. The current WW values for Leaf, Stem, and Shoot are generally higher than those reported for Hanoi, Vietnam, at 5.17 mg/kg (ranging from 4.11-6.01 mg/kg) [7]. Similarly, the current WW concentrations are considerably higher than the 3.9 mg/kg reported for both Thailand [35] and Malaysia [36]. Overall, the current findings from Seremban indicate relatively higher or comparable Zn concentrations in *I. aquatica* tissues compared to many previously reported studies, particularly for DW values, with the root exhibiting a remarkably wide range.

Table 1. Zinc (Zn) concentration in *I. aquatica* tissues and topsoil from Seremban, Negeri Sembilan, Malaysia, compared with previously reported results from other locations. Concentrations are reported as mean (minimum-maximum) in mg/kg DW.

		Weight basis *	Zn Concentration (mg/kg)
Current finding		Leaf (DW)	(52.5 - 562)
		Stem (DW)	(125 - 198)
		Shoot (DW)**	(615-760)
		Root (DW)	(102 - 5080)
		Leaf (WW)	(5.77 - 61.8)
		Stem (WW)	(13.8 - 21.8)
		Shoot (WW)**	(4.70 - 108)
Seremban, Malaysia [30]	2021	(DW)	125 (Leaf); 103 (Stem)
		(WW)	13.4 (Leaf); 10.3 (Stem)
Nal Sarovar Bird Sanctuary, India [31]	2006	(DW)	639.04
Hanoi, Vietnam [7]	2008	(WW)	5.17 (4.11-6.01)
		(DW)	51.5
Thailand [35]			
Malaysia [36]	2004	(WW)	3.9
Malackochha, Sherpur, Bangladesh [33]	2012	(DW)	(157.63-298.64)
University of Malaya, Malaysia [34]	2016	Root (DW)	(1.71-34.70)
		Shoot (DW)	(1.42-35.10)
Hunan, China [32]		(DW)	7.055

*DW=Dry-weight based value; WW=Wet-weight based value; **Shoot=(Leaf+Stem)/2

Current findings on Zn concentrations in *I. aquatica* tissues (Table 1) comparing them with established food safety guidelines (Table 2), a varied picture emerges, particularly when focusing on the wet-weight (WW) basis relevant for food consumption. For the current study, the Zn

concentrations in *I. aquatica* are (5.77 - 61.8) mg/kg WW for Leaf, (13.8 - 21.8) mg/kg WW for Stem, and (4.70 - 108) mg/kg WW for Shoot. When evaluated against the Malaysia Food Regulation (1985) guideline of 100 mg/kg WW, it is observed that the maximum Zn levels in Leaf (61.8 mg/kg

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WW) and Stem (21.8 mg/kg WW) are well within this permissible limit. However, the Shoot concentration, with a maximum of 108 mg/kg WW, can slightly exceed the Malaysian guideline [37]. A stricter threshold is set by the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations (2011) in India, which specifies 50 mg/kg WW [38]. Against this Indian standard, the Stem (max 21.8 mg/kg WW) remains within the safe limit, but the Leaf (max 61.8 mg/kg WW) and particularly the Shoot (max 108 mg/kg WW), demonstrate levels that can significantly surpass this guideline, raising potential concerns for consumers adhering to Indian regulations.

3.2. Metabolite profile of *I. aquatica* leaf tissue

Understanding metabolite changes is crucial for leveraging different Zn forms in agriculture to enhance crop yield and sustainability. By analysing Zn concentrations and speciation, breeders can develop resilient crop varieties adapted to diverse soil conditions and nutrient availability [39]. Among these, ZnO NPs have been shown to improve the uptake of essential nutrients, including Zn, thereby supporting physiological processes and enabling breeding programs to prioritise cultivars that perform well under nanoparticle application. Such responses include increased accumulation of beneficial metabolites, such as flavonoids and amino acids [13]. Tailored nanoparticle formulations, optimised for particle size and concentration, can further promote growth without inducing phytotoxicity, thereby supporting sustainable farming practices with reduced reliance on chemical inputs [40]. Moreover, the ability of ZnO NPs to modulate antioxidant systems and stress responses highlights their potential role in breeding for abiotic stress tolerance (e.g., drought and salinity), ultimately contributing to crop resilience, nutritional quality, and food security [13].

In contrast, long-term exposure to pollutants is often associated with reduced metabolite diversity compared to controls, reflecting a physiological stress response [41, 42]. Under toxic conditions, metabolism shifts towards pathways involved in oxidative stress mitigation and inflammation management, leading to downregulation of other metabolic processes. For example, pollutant-associated metabolites have been linked to inflammatory responses, while others are suppressed as organisms prioritise coping mechanisms [43, 44]. This results in a narrower range of detectable metabolites

in exposed samples.

In the present study, GC-MS analysis of crude methanolic extracts revealed 103–138, 47–114, 88–131, and 77–122 metabolites in the control, ZnSO₄, ZnO, and ZnO NPs treatments, respectively. Mean peak heights (unitless $\pm$ SE) were highest in the control (486,202 $\pm$ 35,993), followed by ZnSO₄ (13,628 $\pm$ 1,170), ZnO (8,873 $\pm$ 962), and ZnO NPs (4,601 $\pm$ 462) (Table 3). A total of 45, 6, 9, and 7 metabolites were consistently detected across replicates in the control, ZnSO₄, ZnO, and ZnO NPs treatments, respectively, and are listed in Table 4 (sections A–D).

The peak height range for the control treatment was 1,898,602 $\pm$ 649,040 (3,6,9,12-tetraoxatetradecan-1-ol) to 24,196 $\pm$ 2,731 (cyclononasiloxane, octadecamethyl-). In the ZnSO₄ treatment, peak heights ranged from 102,182 $\pm$ 6,725 (cyclooctasiloxane, hexadecamethyl-) to 6,054 $\pm$ 943 (4H-pyran-3-carboxylic acid derivative). For ZnO, the range was 152,615 $\pm$ 18,731 (cyclooctasiloxane, hexadecamethyl-) to 3,104 $\pm$ 469 (succinic acid derivative). In ZnO NP-treated samples, values ranged from 45,311 $\pm$ 17,344 (cyclooctasiloxane, hexadecamethyl-) to 2,680 $\pm$ 371 (pyridine-3-carboxamide derivative).

Metabolomic profiling is crucial for understanding plant responses to Zn forms, including ionic Zn²⁺ and ZnO nanoparticles. Alterations were observed in amino acids, organic acids, carbohydrates, and flavonoids, reflecting both core and secondary metabolic adjustments. ZnO NPs induced marked metabolic shifts, enhancing antioxidant defence pathways and promoting the accumulation of beneficial compounds, though high concentrations may impose phytotoxic stress. These findings highlight the potential of ZnO NPs as nanofertilizers, given their ability to alter metabolite synthesis and improve stress resilience, if particle size and dosage are optimised.

This study specifically focused on metabolites consistently detected across replicates, a methodological choice that reduces random variation and experimental noise, thereby improving reliability and robustness of the results [45, 46]. Such metabolites are more likely to reflect genuine physiological responses to ZnO NPs, including alterations in antioxidant activity and stress-related pathways, which have been well-documented in earlier studies [47, 48]. Examining these consistently observed metabolites, therefore provides

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valuable insights into the biochemical pathways impacted by ZnO NPs exposure, offering a stronger basis for understanding plant health and metabolic adaptations [47, 49].

These findings demonstrate that ZnO NPs significantly remodel the metabolome of *I. aquatica*, influencing stress responses, nutrient acquisition, and secondary metabolite production. Understanding these shifts is critical for harnessing nanoparticle-based fertilizers to improve crop resilience, productivity, and sustainability.

3.2.1. Significant metabolite across all treatments: Cyclononasiloxane

Cyclooctasiloxane (hexadecamethyl) and cyclononasiloxane (octadecamethyl) were the only metabolites consistently detected across all treatments. Their persistence in *I. aquatica* indicates a potential role in regulating Zn uptake and tolerance, irrespective of whether Zn was supplied as ZnSO₄, ZnO, or ZnO NPs. The presence of these compounds in control plants suggests that their functions extend beyond heavy metal defence, possibly contributing to baseline physiological or structural processes.

In contrast, cyclohexasiloxane (dodecamethyl) and cyclopentasiloxane (decamethyl) were uniquely associated with ZnO-NP exposure, implying a nanoparticle-specific metabolic response. Siloxanes are known to reduce heavy metal bioavailability by forming soil complexes, thereby limiting uptake [50]. In addition, silicon plays a central role in strengthening antioxidant defences by enhancing the activities of enzymes such as superoxide dismutase, catalase, and peroxidase, which collectively mitigate oxidative stress caused by heavy metal accumulation [51]. Silicon also deposits in cell walls, reinforcing structural integrity and reducing metal-induced cellular injury. At the molecular level, it regulates genes linked to metal transport and stress responses, further improving plant resilience [50].

Cyclooctasiloxane (COS) may also contribute to nutrient dynamics by enhancing the delivery and utilisation of essential elements such as Zn. Since Zn is a cofactor in more than 300 enzymes involved in photosynthesis, protein synthesis, and hormone regulation [52], its availability is crucial for plant growth. Zn exposure itself may stimulate COS synthesis,

which in turn could balance nutrient uptake and mitigate toxicity. Furthermore, Zn's catalytic role in biochemical reactions may indirectly influence siloxane metabolism, potentially accelerating reaction pathways or modulating siloxane chemistry outcomes.

3.2.2. Significant metabolites for ZnO NPs treatment: Silicic acid and pyridine-3-carboxamide

Silicic acid (diethyl bis(trimethylsilyl) ester) and pyridine-3-carboxamide (1,2-dihydro-4,6-dime) were uniquely detected in *I. aquatica* leaves exposed to ZnO NPs.

Silicic acid plays an important role in forming a silica coating around ZnO NPs, which reduces Zn²⁺ dissolution and, consequently, nanoparticle-associated toxicity. This protective layer helps preserve the antimicrobial properties of ZnO while minimising cytotoxic effects [53, 54]. The detection of silicic acid in *I. aquatica* suggests either induction of endogenous synthesis or uptake from the growth environment triggered by ZnO NPs exposure, a mechanism that warrants further investigation.

Pyridine-3-carboxamide, a widely occurring heterocyclic compound, is a known constituent of vitamin B₆ and several alkaloids [55]. It exhibits diverse biological activities, including antiviral [56], antibacterial, antifungal [57, 58], pesticidal, herbicidal [58], antiprotozoal, nematocidal, antitubercular, anticancer [59], local anaesthetic, anticonvulsant, antioxidant, anti-inflammatory, and potential antimycotic effects [60].

The exclusive detection of pyridine-3-carboxamide in leaves exposed to 50 mg/L ZnO NPs highlights a possible role of ZnO in stimulating its biosynthesis. Given their catalytic properties, ZnO NPs may alter plant metabolic pathways, thereby facilitating the production of such secondary metabolites [61]. However, ZnO NPs exposure can also induce phytotoxic effects, particularly oxidative stress, underscoring the importance of carefully balancing nanoparticle concentration with plant physiological responses. While ZnO NPs can enhance secondary metabolite production, optimising their application is essential to maximise benefits while minimising potential toxicity [62].

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Table 2. Guidelines on Zn concentrations for food safety established by different regulatory organisations.

Regulatory authority (Country)	Zn concentration (mg/kg WW)	Reference
Malaysia Food Regulation (1985) (Malaysia)	100 mg/kg WW	[37]
Food safety standard (contaminants, toxins, and residues) regulations (2011) (India)	50 mg/kg WW	[38]

Table 3. Mean peak height, along with the standard error (SE), of the metabolite profile in the leaf tissue of *I. aquatica* was assessed following exposure to 50 mg/L of ZnSO₄, ZnO, and ZnO NPs.

	Metabolites detected	Mean peak height	±	SE
Ctrl	103-138	486202	±	35993
ZnSO ₄	47-114	13628	±	1170
ZnO	88-131	8873	±	962
ZnO NPs	77-122	4601	±	462

Table 4. Major metabolites detected (mean signal height±SE) in (a) control treatment, (b) ZnSO₄, (c) ZnO, and (d) ZnO NPs in leaf of *I. aquatica*.

(a) Control treatment				
		Height	±	SE
1	3,6,9, 12-Tetraoxatetradecan-1-01	1,898,602	±	649,040
2	Hexagol	1,644,996	±	859,616
3	1,4-Dioxaspiro [4.5] decane-7-butanoic acid, 6-1	1,482,416	±	974,357
4	Hexadecanoic acid, 2-hydroxyethyl ester	1,379,655	±	792,326
5	Hexadecanoic acid, methyl ester	1,301,339	±	564,151
6	Stearic acid, 2-hydroxy-1-methylpropyl ester	1,256,496	±	509,176
7	Oleoyl chloride	1,112,485	±	421,918
8	Octadecanoic acid, 2-hydroxyethyl ester	1,103,204	±	751,646
9	Ethylene brassylate	1,084,268	±	652,991
10	Hexanoic acid, 2-ethoxyethyl ester	910,352	±	359,260
11	Ethyl Oleate	889,939	±	660,374
12	2-[2-[2-[2-(2-Methoxyethoxy) ethoxy] ethox.	840,571	±	337,887
13	Triethylene glycol monododecyl ether	828,529	±	284,256
14	cis-10-Nonadecenoic acid	723,105	±	299,846
15	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl	709,106	±	261,277
16	Octadecanoic acid, ethyl ester	632,418	±	402,030
17	Heptaethylene glycol monododecyl ether	611,170	±	198,943
18	Octadecanoic acid, 2-(octadecyloxy)ethyl ester	591,620	±	230,587
19	Oleic acid, 3-hydroxypropyl ester	562,390	±	381,777
20	1,4,7,10,13,16-Hexaoxacyclooctadecane	523,275	±	245,725
21	3,6,9,12-Tetraoxatetradecan-1-01	455,288	±	160,738
22	2,5,8,11, 14-Pentaoxahexadecan-16-01	425,416	±	271,903
23	Hexadecanoic acid, ethyl ester	416,904	±	283,502
24	8-Heptadecene	399,863	±	214,297
25	Cyclohexyl-15-crown-5	359,312	±	135,688
26	Octaethylene glycol monododecyl ether	357,062	±	174,429
27	tert-Butyl-(2-ethoxyethoxy) dimethylsilane	330,660	±	206,532
28	Hexaethylene glycol monododecyl ether	288,492	±	186,466
29	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,	287,693	±	106,778

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Table 4. Major metabolites detected (mean signal height $\pm$ SE) in (a) control treatment, (b) ZnSO₄, (c) ZnO, and (d) ZnO NPs in leaf of *I. aquatica*. (Continued)

30	2,2'-Bi-1,3-dioxolane	253,024	$\pm$	123,447
31	Oleic acid, 2-hydroxyethyl ester stearate	251,227	$\pm$	89,038
32	1 -Dimethyl(ethenyl)silyloxytetradecane	222,763	$\pm$	128,226
33	7-Hexadecenoic acid, methyl ester, (Z)-	217,685	$\pm$	112,657
34	1,4,7,10,13,16, 19-Heptaosa-2-cycloheicosal2	203,359	$\pm$	70,282
35	Tetraethylene glycol diethyl ether	189,567	$\pm$	65,738
36	2-Nonadecanone	184,864	$\pm$	86,874
37	Hexadecane	170,872	$\pm$	87,750
38	1 -Hexadecanol	126,428	$\pm$	62,180
39	Isobutyric acid, tridecyl ester	101,151	$\pm$	46,176
40	Palmitic acid vinyl ester	85,371	$\pm$	57,109
41	Cyclooctasiloxane, hexadecamethyl-	63,285	$\pm$	21,467
42	erythro-9, 10-Dibromopentacosane	58,374	$\pm$	24,078
43	1,1,1,5,7,7,7-Heptamethyl-3 ,3 -bis (trimethylsil)	51,783	$\pm$	18,979
44	Pentadecanal-	42,174	$\pm$	11,749
45	Cyclononasiloxane, octadecamethyl-	24,196	$\pm$	2,731
(b) ZnSO₄				
		Height	$\pm$	SE
1	Cyclooctasiloxane, hexadecamethyl-	102,182	$\pm$	6,725
2	Cyclohexasiloxane, dodecamethyl-	40,577	$\pm$	2,920
3	1,1,1,5,7,7,7-Heptamethyl-3 ,3 -bis (trimethylsil (	22,803	$\pm$	3,485
4	Cyclononasiloxane, octadecamethyl-	17,589	$\pm$	2,199
5	Cyclopentasiloxane, decamethyl-	6,233	$\pm$	1,088
6	4H-Pyran-3-carboxylic acid, 6-amino-4-(3-bromo-4-fluorophenyl)-5-cyano-2-phenyl-, ethyl ester	6,054	$\pm$	943
(c) ZnO				
		Height	$\pm$	SE
1	Cyclooctasiloxane, hexadecamethyl-	152,615	$\pm$	18,731
2	Cyclohexasiloxane, dodecamethyl-	57,755	$\pm$	5,659
3	Cyclononasiloxane, octadecamethyl-	14,301	$\pm$	1,859
4	Cyclopentasiloxane, decamethyl-	12,954	$\pm$	2,982
5	Cyclopentasiloxane, decamethyl	12,410	$\pm$	1,819
6	1 -(4-Acetamidoanilino)-3 ,7-dimethylbenzo [4,5 [ZnO only]	6,608	$\pm$	3,545
7	Cyclotetrasiloxane, octamethyl-	5,308	$\pm$	914
8	Spiro(1,3-dioxolane)-2,3'-pregn-5'-en-20'-ol, 11	3,313	$\pm$	268
9	Succinic acid, bis (2,2,3,3,4,4,5,5,6,6,7,7-dodec [ZnO only]	3,104	$\pm$	469
(d) ZnO NPs				
		Height	$\pm$	SE
1	Cyclooctasiloxane, hexadecamethyl- [4]	45,311	$\pm$	17,344
2	Cyclohexasiloxane, dodecamethyl- [3] xcontrol	26,917	$\pm$	5,691
3	Cyclopentasiloxane, decamethyl- [3] no control	9,402	$\pm$	2,294
4	Cyclononasiloxane, octadecamethyl- ALL	8,233	$\pm$	2,144
5	Cyclotetrasiloxane, octamethyl- [2] ZnO and ZnO NPs	4,267	$\pm$	471
6	Silicic acid, diethyl bis(trimethylsilyl) ester [1]	2,944	$\pm$	312
7	Pyridine-3-carboxamide, 1 ,2-dihydro-4,6-dime [1]	2,680	$\pm$	371

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4. Conclusion

This study investigates the effects of hydroponic exposure to 50.0 mg/L ZnSO₄, ZnO, and ZnO NPs on *I. aquatica*, demonstrating that the type of Zn significantly influences Zn accumulation, growth responses, and metabolite profiles. Zn distribution throughout the plant showed roots as the primary site of accumulation, with ZnSO₄ leading to an exceptionally high root accumulation of 4620 mg/kg DW. The study also observed a clear growth hierarchy (control > ZnO > ZnO NPs > ZnSO₄). These findings reveal complex metal interactions and biochemical adaptations, such as drastic stress-induced reductions in metabolite diversity. GC-MS analysis showed that the total number of detected metabolites ranged from 103-138 for the control, 47.0-114 for ZnSO₄, and 77.0-122 for ZnO NPs. More specifically, the number of *common metabolites* identified decreased sharply from 45.0 in the control to 6.00 in ZnSO₄, 9.00 in ZnO, and 7.00 in ZnO NPs treatments. The overall mean peak height also exhibited a substantial decrease, from 486000 ± 36000 in the control to as low as 4600 ± 462 in ZnO NP-treated plants. The unique presence of Silicic acid (diethyl bis(trimethylsilyl) ester) and Pyridine-3-carboxamide (1,2-dihydro-4,6-dime) in ZnO NP-treated plants is particularly noteworthy. Silicic acid's detection suggests its role in forming a silica coating around ZnO NPs, which can reduce Zn²⁺ dissolution and associated toxicity. Pyridine-3-carboxamide, a compound with diverse biological activities including antiviral, antibacterial, and anticancer properties, indicates that ZnO NPs may alter plant metabolic pathways or promote *I. aquatica* as a biosynthetic platform. These specific biochemical adaptations offer crucial insights into the phytoremediation potential of *I. aquatica* for heavy metal-contaminated environments and provide strategies for optimising micronutrient management in agriculture. However, the variability in plant responses across species and the intricate dynamics of metal interactions underscore the need for further research to fully harness these benefits for sustainable environmental and agricultural solutions.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Koe Wei Wong under the close supervision of Chee Kong Yap, Rosimah Nulit, Hishamuddin Omar, Ahmad Zaharin Aris, Yoshifumi Horie, Meng Chuan Ong and Wan Mohd Syazwan. The first draft of the manuscript was written by Koe Wei Wong and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declaration of generative AI and AI-assisted technologies in the writing process

ChatGPT and Gemini were employed as tools to refine language and readability during the writing process. However, the authors conducted a thorough review and editing of the content, thereby accepting complete responsibility for the publication's content, generation, and summarisation.

Conflicts of interest

The authors declare that there is no conflict of interest among them. Chee Kong Yap, as an Editor of this journal, didn't participate in the peer review, editorial handling, or decision making of this manuscript. Full responsibility for the editorial process was under the supervision of the Editor-in-Chief.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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