

Integrated response to lead in saline aquaponics: plant-based remediation, microbial community shifts, and shrimp health

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Abstract

This study aimed to evaluate the multiscale biological responses to subchronic lead (Pb) exposure in a saline aquaponic system, using *Sesuvium portulacastrum* and *Litopenaeus vannamei* as model organisms. Physiological, biochemical, and microbiological indicators were assessed to characterize the impacts of dissolved Pb. *S. portulacastrum* demonstrated high Pb retention efficiency, exceeding 90% and achieving complete removal in certain weeks. However, retention fluctuated over time, modulated by nutrient dynamics, especially ammonium and phosphorus levels, suggesting ionic competition and phosphate precipitation as factors influencing metal bioavailability. Pb accumulated in all plant tissues, with patterns indicating active translocation from roots to aerial parts, and triggered a complex antioxidant response, characterized by dynamic changes in peroxidase and catalase activity. In *L. vannamei*, Pb bioaccumulated predominantly in the cephalothorax, causing metabolic disruptions, including elevated hemolymph protein and lipid levels, alongside marked immunosuppression. Reductions in hemocyte counts, lysozyme activity, and NBT reduction confirmed compromised immune and oxidative function, while catalase activity increased as a potential compensatory mechanism. Rhizospheric microbiota of Pb-exposed plants exhibited significant structural shifts, with increased alpha diversity and taxonomic enrichment of metal-tolerant genera such as *Neptunomonas*, *Ferrimonas*, and *Arcobacter*. These genera were strongly correlated with physiological and enzymatic stress indicators, supporting their role as functional microbial biomarkers of Pb exposure. Our findings highlight the multidimensional effects of lead in aquaponics, impacting plant physiology, shrimp health, and microbial ecology. This integrated evaluation provides a robust framework for microbiome-assisted phytoremediation strategies and the development of more resilient, metal-tolerant aquaponic systems.

Introduction

Heavy metal contamination in aquatic environments, particularly by lead (Pb), poses a serious threat to public health, food safety, and the sustainability of productive systems (Khedr and Ghannam 2025). The Pacific white shrimp (*Litopenaeus vannamei*), a commercially valuable species, is especially susceptible to Pb bioaccumulation, which compromises its safety for human consumption. Concurrently, halophytic plants such as *Sesuvium portulacastrum* have gained attention for the phytoremediation of saline environments contaminated with heavy metals, due to their strong tolerance, antioxidant capacity, and ability to accumulate metals without impairing growth (Wang et al. 2014; Alsherif et al. 2023).

In intensive systems like aquaponics, Pb can recirculate and concentrate within the closed loop, accumulating in cultivated organisms and posing toxicological risks both to human health and to the ecological stability of the system. Although Pb concentrations reported in farmed shrimp are generally below the FAO/WHO permissible limit (Martínez et al. 2024), the World Health Organization warns that no level of Pb exposure is considered safe, as it can accumulate in bone tissue with a half-life of up to 30 years (WHO 2024). This makes contaminant monitoring and the development of effective removal strategies imperative, without compromising aquaculture productivity.

S. portulacastrum has shown promising phytoremediation potential in hydroponic systems, accumulating high levels of metals such as Cd, Ni, and Zn (He et al. 2022), through mechanisms including vacuolar compartmentalization, the expression of metal-binding proteins, and the synthesis of antioxidant compounds (Sharma et al. 2016). Notably, its ability to produce osmoprotectants and activate specific metabolic pathways enables it to tolerate high salinity and chemical stress. These features, along with its vigorous growth under semi-controlled conditions, position it as a strong candidate for integrated remediation systems.

Beyond its intrinsic physiological mechanisms, the rhizospheric microbiome associated with *S. portulacastrum* plays a key role in heavy metal detoxification. Various microorganisms in the rhizosphere contribute to Pb immobilization through biosorption to exopolysaccharides, enzyme-mediated precipitation, phosphate solubilization, or pH modification (Barra Caracciolo and Terenzi 2021; Zhou et al. 2024). The manipulation of these microbial communities—via engineered

consortia or natural selection—has been shown to significantly enhance phytoremediation efficiency in contaminated environments (Imran et al. 2025).

From an animal health perspective, *L. vannamei* serves as a sensitive bioindicator of metal pollution due to its high filtration rate, rapid metabolism, and well-characterized immune responses. Studies have shown that Pb concentrations as low as 0.1 mg/L can reduce hemocyte counts by over 40% and impair the production of reactive oxygen species (Wu et al. 2017). Disruption of osmoregulatory capacity has also been reported (Usman et al. 2013), along with elevated hepatic biomarkers, which are associated with cellular damage and metabolic stress (Baruch-Garduza et al. 2022; Zhang et al. 2023). These alterations reflect a systemic impact that compromises both physiological stability and immunocompetence.

In this context, integrated approaches are needed that simultaneously consider water quality, plant responses, aquatic animal health, and microbiome functionality. Aquaponics, due to its closed nature and high resource efficiency, offers an ideal platform for testing sustainable and scalable remediation strategies. The present study aimed to comprehensively assess the effects of Pb in a saline aquaponic system. The phytoremediation efficiency of *S. portulacastrum* was quantified over a 12-week subchronic exposure period, while Pb accumulation was evaluated in both plant and shrimp tissues. Oxidative stress and immune biomarkers were measured in *L. vannamei*, and the functional structure of the rhizospheric microbiome was characterized through metataxonomic analysis of the 16S rRNA V3–V4 region, including alpha and beta diversity metrics, differential abundances, and functional correlations with plant biochemical variables.

We hypothesized that *S. portulacastrum*, in interaction with its rhizospheric microbiota, could reduce dissolved Pb concentrations in the system by at least 50%, resulting in lower Pb bioaccumulation in *L. vannamei* and attenuated immunotoxic effects. This multiscale approach—integrating plant physiology, animal health, and microbial ecology—aims to support the design of safer, more resilient aquaponic production systems capable of withstanding heavy metal stress.

Materials and methods

Study design and experimental context

This research was conducted as an observational study aimed at examining the multiscale biological and ecological responses to chronic lead (Pb) exposure within a saline aquaponic system. The seawater used in the system was extracted from a coastal deep well (95 meters depth), with an average salinity of 29 PSU and a lead concentration of $3.01 \pm 1.03 \mu\text{g/L}$. Although no artificial Pb dosing was applied, the presence of naturally elevated lead levels, likely of geogenic origin, provided a consistent exposure scenario representative of coastal groundwater systems influenced by subsurface metal mobilization.

A comprehensive eight-week monitoring was carried out to evaluate Pb bioaccumulation and physiological responses in the system's biotic components. Weekly sampling of water, plants (*S. portulacastrum*), and shrimp (*L. vannamei*) enabled a temporal analysis of lead dynamics and biological impacts across trophic levels. As a reference, unexposed control organisms—both plants and shrimp—were collected from natural sites with no known Pb contamination and maintained under comparable recirculating conditions. This design allowed for a holistic evaluation of chronic metal toxicity under environmentally relevant, yet non-anthropogenic, exposure conditions.

All procedures involving live animals adhered to the Mexican standard NOM-033-SAG/ZOO-2014, as well as international guidelines for the ethical treatment of aquatic organisms in research, ensuring minimal stress and humane handling throughout the study.

Experimental model

The study was conducted using a nutrient film technique (NFT) aquaponic system located in Mérida, Yucatán (21°05'31"N, 89°62'38"W). The system consisted of a circular aquaculture tank with a capacity of 1.05 m³, connected to four PVC pipes measuring 7.5 cm in diameter and 1.13 m in length, which made up the hydroponic component. Each pipe had six perforations spaced 8 cm apart to accommodate the plants. Water flow originated from the aquaculture tank and descended by gravity into a sedimentation chamber filled with 2 kg of randomly arranged short PVC tubes, which acted as a medium for flow deceleration and solid capture. From there, water was directed to a second chamber equipped with a submersible CROC inverter pump (C5000, Denderleeuw, Belgium), which delivered a flow rate of 5000 L/h to a biofilter. This chamber functioned as a hydraulic transition or buffer zone to ensure a more stable flow into the biofiltration unit. The biofilter contained 1.28 kg of Kaldnes Bio Filter media (K1), with a biofiltration volume of 0.14 m³, designed to promote nitrification. Finally, the water flowed again by gravity through the hydroponic pipes and returned to the aquaculture tank, thus completing the recirculation cycle. Water flow through the hydroponic pipes was maintained under laminar conditions, with an estimated velocity of 1 to 3 L/min per channel, generating a thin film of water (~ 2–3 mm) suitable for root oxygenation and efficient nutrient uptake.

Aquaponic species

For this study, 66 shrimp with an average weight of 35 ± 5.9 g were stocked at a density of 2.2 kg/m³. Feeding was carried out twice daily, with rations adjusted between 1.0 and 1.5% of the shrimp's body weight, depending on their size. A commercial diet formulated for the fattening stage was used, containing 30% protein and 6–8% lipids.

As the plant species, 24 cuttings of dune purslane (*S. portulacastrum*) were collected from their natural habitat in coastal dunes. Each cutting was individually placed in hydroponic baskets inserted into the holes of the NFT system and acclimated for three weeks within the aquaponic setup. Vegetable sponge was used as the substrate to provide initial support for the cuttings and ensure proper root aeration. Additionally, a metal mesh with 10 × 10 cm openings was installed over the NFT pipes to serve as a support structure for the plant's creeping growth, promoting natural development without obstructing water flow or access to other plants.

To validate the experiment, control groups of plants and animals not exposed to lead were included. The plant control group consisted of *S. portulacastrum* specimens from their natural dune habitat. Although these plants may contain trace amounts of environmentally sourced lead, it is important to note that such an ecosystem is not subject to controlled recirculation conditions or defined sources of contamination as in the experimental system. The animal control group comprised shrimp cultivated in ponds supplied with seawater, in which no deliberate lead exposure occurred. However, it is acknowledged that these organisms may have been exposed to residual levels of lead inherent to the marine environment, albeit under different conditions of hydrodynamics, accumulation, and bioavailability compared to the experimental setup.

Water chemical quality

To monitor variability in water quality, weekly analyses were conducted using spectrophotometric techniques (Hach DR 2800, Loveland, CO, USA). Un-ionized ammonia nitrogen (NH₃-N) was quantified using the salicylate-hypochlorite method (Hach No. 8155); nitrite (NO₂-N) was determined using the sulfanilamide method in acidic solution (Hach No. 8507); and nitrate (NO₃-N) was measured after reduction to NO₂-N using a copper-cadmium column (Hach No. 8039). Total phosphorus (TP) was assessed by acid-persulfate digestion (Hach No. 8190), and potassium (K⁺) was quantified using the tetraphenylborate method (Hach No. 8049).

Quantification of lead (Pb) in water and biological tissues

Lead concentrations were quantified by visible absorption spectrophotometry (DR 2800, Hach, USA) using Hach Method 10083 (5-Carboxy-PADAP), with a detection range of 1–200 µg/L. Absorbance was read at 548 nm, corresponding to the Pb–PADAP complex. Shrimp tissue (1 g, homogenized) was digested with 10 mL of 65% HNO₃ at 60–95°C until complete dissolution, followed by 2 mL of H₂O₂ to remove organic matter, following AOAC Method 999.10. After cooling, Pb content was measured spectrophotometrically and expressed as µg/g tissue. For water analysis, triplicate 200 mL samples were collected at the biofilter and outlet points, analyzed directly—without digestion—using the same method. Results were expressed as µg/mL of water.

Shrimp assays

Hemolymph was extracted from the ventral sinus using sterile syringes and mixed 1:1 with an anticoagulant solution (450 mM NaCl, 10 mM EDTA, 30 mM sodium citrate, pH 7.3) to prevent coagulation and preserve cellular integrity. Samples were kept on ice and analyzed immediately. Osmolarity was measured using a vapor pressure osmometer (Wescor Vapro® 5520), and total hemocyte count (THC) was performed by mixing 50 µL of hemolymph with anticoagulant solution and counting cells under a light microscope in a Neubauer chamber.

Indirect markers of oxidative and immune stress—reactive oxygen species (ROS), catalase (CAT), and lysozyme (LZM)—were also analyzed. ROS production was measured via NBT reduction following Song and Hsieh (1994). CAT activity was determined based on H₂O₂ decomposition (Aebi 1984), and LZM activity was evaluated using a turbidimetric assay with *Micrococcus lysodeikticus* (Mörsky 1983). Absorbance measurements were performed using an ELISA plate reader and a UV-Vis spectrophotometer as appropriate.

The biochemical composition of hemolymph was assessed in triplicate, quantifying total protein (TP), carbohydrates (CBO), and cholesterol (CHO), expressed in mg/mL. TP was determined via the Lowry method (Lowry et al. 1951), CBO by the phenol-sulfuric acid method (Dubois et al. 1956), and CHO by the Watson method (Watson 1960).

Antioxidant response of *Sesuvium portulacastrum*

To analyze antioxidant components, stems, and leaves of *S. portulacastrum* were ground in liquid nitrogen and subjected to two extraction protocols. Non-protein antioxidants (lycopene, ascorbic acid) were extracted using a HEPES/NaOH buffer (Bonfig et al. 2010), while protein-based enzymes (POD, CAT, APX) were extracted with potassium phosphate buffer. Supernatants were stored at –20°C, and protein content was determined by the Lowry method (Lowry et al. 1951).

Peroxidase (POD) activity was measured at 470 nm following Chance and Maehly (1955), catalase (CAT) at 240 nm per Aebi (1984), and ascorbate peroxidase (APX) at 290 nm following Nakano and Asada (1981). Lycopene content (LYP) was quantified via hexane-acetone extraction and absorbance at 502 nm, according to Bunghez et al. (2011). Ascorbic acid (AA) was determined by spectrophotometric quantification at 265 nm, based on the DTT/ascorbate oxidase method described by Foyer (2017).

Root microbiome analysis of *S. portulacastrum* via 16S rRNA sequencing

Root samples of *S. portulacastrum* cultivated in a lead-exposed aquaponic system were compared to those collected from a natural coastal dune ecosystem. Two experimental conditions were analyzed: three biological replicates of Pb-exposed plants (Pb-S) and three replicates of unexposed control plants (Control). Roots were immediately frozen in liquid nitrogen and ground into a fine powder. Total genomic DNA was extracted using the Quick-DNA™ Soil Microbe Miniprep Kit (Zymo Research, Cat. No. D6010), following the manufacturer's instructions. The protocol involved mechanical lysis via bead beating, chemical lysis, centrifugation-based separation, and silica column purification. DNA concentration was

determined using a Nanodrop™ 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and integrity was confirmed by 1.5% agarose gel electrophoresis.

Bacterial community profiling was performed by high-throughput sequencing of the 16S rRNA gene, targeting the V3–V4 hypervariable region, commonly used for metataxonomic studies. Amplification was carried out using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). Libraries were prepared using the Quick-16S™ NGS Library Prep Kit (Zymo Research) and sequenced on an Illumina MiSeq™ platform using the v3 600-cycle kit, generating high-resolution paired-end reads ($\sim 2 \times 300$ bp).

Microbiome data analysis

Raw sequencing data were processed with the DADA2 pipeline for quality filtering, error correction, and inference of high-resolution amplicon sequence variants (ASVs), offering greater taxonomic resolution than OTU-based methods. Low-quality and chimeric reads were removed. ASVs were classified using the ZymoBIOMICS® database and validated via BLAST against NCBI. An ASV abundance matrix was generated for downstream analyses of diversity, community structure, and taxon-specific patterns.

Alpha diversity was assessed using Shannon and Simpson–Evenness indices, with group differences tested by Kruskal–Wallis. Beta diversity was analyzed via Bray–Curtis dissimilarity and visualized with multidimensional scaling (MDS); although MDS plots showed separation, PERMANOVA ($p = 1.0$) found no significant differences. Venn diagrams identified shared and unique genera between treatments.

A Random Forest model trained on relative abundance data identified the top 10 genera most predictive of Pb exposure as potential microbial biomarkers. Spearman correlation heatmaps were generated to explore links between bacterial taxa and plant biochemical markers (e.g., Pb content, antioxidant enzyme activity).

Statistical data analysis

Descriptive data for biometric, physiological, biochemical, immunological, microbiological, and water quality variables were expressed as mean $\pm$ SE. Normality and homogeneity were verified using the Shapiro–Wilk and Levene's tests. Two-way ANOVAs were applied to evaluate the effects of treatment, time, and their interaction on shrimp hemolymph and plant tissue variables. For Pb accumulation, ANOVAs assessed the effects of treatment, tissue type, and exposure duration. Spearman correlations were used to explore relationships between water quality and Pb retention, with the most significant predictors incorporated into a multiple regression model. Statistical significance was set at $p < 0.05$. Analyses were performed using XLSTAT 2023.1 (Addinsoft, Paris, France).

Results

Interactions between water nutrients and lead dynamics in the aquaponic system

Weekly measurements at the inlet and outlet of the hydroponic unit with *S. portulacastrum* showed a consistent decrease in Pb concentrations at the outlet, indicating effective retention by the plants (Table 1). The system displayed high phytoremediation efficiency, reaching 100% in week 6. Weeks 1, 2, and 5 also showed high retention (66–75%), while week 3 dropped to 36.4%, possibly due to environmental or physiological limitations. In week 7, an increase at the outlet may reflect system saturation under high inlet Pb, although final levels remained below initial inputs.

Table 1
Weekly lead retention (%) between inlet and outlet of the hydroponic system with *Sesuvium portulacastrum*. Values are mean $\pm$ S.E. (n = 5).

Week	Inlet Pb (ug/L)	Outlet Pb (ug/L)	Pb-Retained (%)
1	4.00 $\pm$ 0.23	1.00 $\pm$ 0.06	75.0
2	6.00 $\pm$ 0.35	1.50 $\pm$ 0.09	75.0
3	5.50 $\pm$ 0.32	3.50 $\pm$ 0.20	36.4
4	8.50 $\pm$ 0.49	0.50 $\pm$ 0.03	94.1
5	3.00 $\pm$ 0.17	1.00 $\pm$ 0.06	66.7
6	2.50 $\pm$ 0.14	0.00 $\pm$ 0.00	100
7	13.00 $\pm$ 0.75	4.00 $\pm$ 0.23	69.2
8	8.00 $\pm$ 0.46	1.00 $\pm$ 0.06	87.5

Throughout the experiment, nutrient concentrations (NH_4^+ , NO_2^- , NO_3^- , TP, K^+) fluctuated between inlet and outlet. Nitrate levels were generally higher at the outlet—up to 45.2 mg/L in week 6 vs. 31.7 mg/L at the inlet—indicating active nitrification and nutrient availability. Ammonium consistently decreased at the outlet (3.8 to 0.4 mg/L), suggesting microbial transformation and possible plant uptake. Occasional nitrite peaks (e.g., 3.1 mg/L in week 3) suggest partial nitrification, while nitrate accumulation supports complete microbial conversion of NH_4^+ to NO_2^- and then to NO_3^- . Supplementary Table S1 provides full nutrient profiles.

Correlation analysis revealed that inlet NH_4^+ and TP were negatively associated with Pb retention ($R = -0.55$ and -0.49), likely due to ionic competition or Pb–phosphate complex formation. In contrast, outlet NH_4^+ was positively correlated ($R = 0.42$), possibly reflecting more favorable uptake conditions. A multiple regression model based on these variables explained 55.5% of the variation in Pb retention ($R^2 = 0.555$), highlighting the influence of nutrient dynamics on metal removal efficiency.

$$\text{Pb Retained} = 145.42 - 246.37 \times \text{NH}_4^+_{\text{In}} - 7.96 \times \text{TP}_{\text{In}} + 57.82 \times \text{NH}_4^+_{\text{Out}}$$

This equation indicates that higher NH_4^+ at the inlet ($\text{NH}_4^+_{\text{In}}$) are associated with reduced Pb retention, as evidenced by a strong negative coefficient (-246.37). In contrast, increased NH_4^+ levels at the outlet ($\text{NH}_4^+_{\text{Out}}$) are positively associated with Pb retention ($+57.82$). Elevated levels of TP at the inlet (TP_{In}) also exert a negative effect (-7.96), possibly due to the formation of insoluble lead-phosphate complexes in the nutrient solution.

Weekly variation in lead accumulation in leaf, stem, and root of *S. portulacastrum*

Table 2 shows the concentration of Pb in leaf, stem, and root tissues of *S. portulacastrum* over eight weeks of experimental exposure. These values were compared with samples collected from their natural habitat (coastal dune). Pb concentrations varied over time in all plant parts. In leaves, notable increases were observed in weeks 4, 7, and 8, peaking at 197.88 $\mu\text{g}/100\text{ g}$ in week 8. In stems, Pb levels fluctuated, reaching a maximum of 243.34 $\mu\text{g}/100\text{ g}$ in week 5. Root concentrations showed less pronounced variation, with a decrease in Week 5 (71.89 $\mu\text{g}/100\text{ g}$), followed by a progressive increase in weeks 7 and 8 (131.58 and 136.84 $\mu\text{g}/100\text{ g}$, respectively).

Table 2
Lead concentrations ($\mu\text{g}/100\text{ g}$) in leaf, stem, and root of Pb-exposed and control *S. portulacastrum* across eight weeks. Data are mean $\pm$ S.E. (n = 5).

Week	Pb-Exposed			Control		
	Leaf	Stem	Root	Leaf	Stem	Root
1	132.51 $\pm$ 12.8	109.97 $\pm$ 6.3	176.02 $\pm$ 10.2	16.47 $\pm$ 1.0	11.79 $\pm$ 0.7	11.06 $\pm$ 0.6
2	94.32 $\pm$ 5.4	71.48 $\pm$ 4.1	143.00 $\pm$ 8.3	27.75 $\pm$ 2.6	11.34 $\pm$ 0.8	16.09 $\pm$ 0.9
3	126.71 $\pm$ 17.3	98.49 $\pm$ 5.1	136.84 $\pm$ 10.9	44.31 $\pm$ 1.8	11.03 $\pm$ 0.8	16.49 $\pm$ 1.0
4	160.92 $\pm$ 9.3	202.78 $\pm$ 11.7	65.13 $\pm$ 3.8	22.21 $\pm$ 1.3	16.53 $\pm$ 1.0	16.13 $\pm$ 0.9
5	37.77 $\pm$ 2.2	243.34 $\pm$ 14.0	71.89 $\pm$ 4.2	22.03 $\pm$ 1.3	5.49 $\pm$ 0.3	22.32 $\pm$ 1.3
6	48.05 $\pm$ 2.8	53.59 $\pm$ 3.1	82.95 $\pm$ 4.8	11.04 $\pm$ 0.9	16.65 $\pm$ 1.0	16.59 $\pm$ 1.0
7	182.74 $\pm$ 10.6	132.34 $\pm$ 7.6	131.58 $\pm$ 9.6	10.94 $\pm$ 0.6	5.48 $\pm$ 0.3	16.79 $\pm$ 1.0
8	197.88 $\pm$ 11.4	102.98 $\pm$ 5.9	136.84 $\pm$ 7.9	11.04 $\pm$ 0.9	5.50 $\pm$ 0.3	22.12 $\pm$ 1.2

In contrast, plants from the control group showed significantly lower Pb concentrations in all tissues. Leaf values remained low throughout the study (11.04 to 44.31 $\mu\text{g}/100\text{ g}$), with similar trends in stems (5.48 to 16.53 $\mu\text{g}/100\text{ g}$) and roots (5.48 to 22.32 $\mu\text{g}/100\text{ g}$). ANOVA was conducted to assess the effects of treatment (Pb-S vs. control), tissue type (leaf, stem, root), and time (weeks 1 to 8) on Pb accumulation. A significant effect of treatment was detected ($p < 0.001$), indicating that Pb exposure in the aquaponic system led to substantially higher accumulation than in control plants. However, no significant effects were found for time ($p = 0.970$) or tissue type ($p = 0.986$), suggesting that Pb concentrations did not vary consistently over time or between plant parts. Despite this, the observed fluctuations across tissues may reflect differential capacities for Pb uptake, translocation, and storage throughout the experimental period.

Weekly variation in lead accumulation in tissues of *L. vannamei*

Table 3 presents lead concentrations in the tail muscle and cephalothorax of *L. vannamei* over eight weeks of experimental exposure. Shrimp exposed to lead exhibited significantly higher concentrations in both tissues compared to the control group, whose levels remained near zero for most of the study period. In all weeks analyzed, lead concentrations were consistently higher in the cephalothorax than in the tail muscle, reaching maximum values exceeding 2400 $\mu\text{g}/100\text{ g}$. This supports the hypothesis of preferential accumulation in the cephalothorax.

Table 3
Lead concentrations ($\mu\text{g}/100\text{ g}$) in tail muscle and cephalothorax by week in lead-exposed and control shrimp (Data are mean $\pm$ S.E.; n = 3)

Week	Pb-Exposed		Control	
	Tail muscle	Cephalothorax	Tail muscle	Cephalothorax
1	88.61 $\pm$ 5.12	721.12 $\pm$ 41.63	0.00 $\pm$ 0.00	25.17 $\pm$ 1.45
2	356.54 $\pm$ 20.58	785.75 $\pm$ 43.25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
3	184.05 $\pm$ 10.63	667.36 $\pm$ 36.25	36.90 $\pm$ 2.13	14.10 $\pm$ 0.87
4	218.79 $\pm$ 12.56	998.32 $\pm$ 58.13	0.00 $\pm$ 0.00	28.41 $\pm$ 1.65
5	186.87 $\pm$ 10.98	1998.03 $\pm$ 115.24	44.41 $\pm$ 3.58	26.78 $\pm$ 1.55
6	133.44 $\pm$ 7.70	1996.58 $\pm$ 106.32	20.69 $\pm$ 1.18	29.03 $\pm$ 1.72
7	115.54 $\pm$ 6.67	2407.64 $\pm$ 142.35	37.63 $\pm$ 2.18	0.00 $\pm$ 0.00
8	263.87 $\pm$ 15.23	1993.69 $\pm$ 119.85	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

Weekly fluctuations, particularly in the tail muscle, may reflect individual variation in physiological processes such as absorption, detoxification, or excretion, as well as changes in metal bioavailability during the experiment. Conversely, persistently low levels in the control group confirm the absence of significant external contamination throughout the trial. A factorial ANOVA revealed significant effects of both treatment (Pb exposure) and tissue type on lead concentration ($p = 0.0004$ and $p = 0.0007$, respectively). A significant interaction between treatment and tissue type was also detected ($p = 0.0049$), indicating that the distribution of lead among tissues depends on the exposure condition. In contrast, the factor "week" ($p = 0.824$) and its interactions with treatment or tissue type showed no significant effects ($p > 0.78$), suggesting that lead concentrations did not follow a consistent temporal pattern within each group.

Post hoc analysis confirmed that the cephalothorax of Pb-exposed shrimp had significantly higher lead concentrations than all other groups ($p < 0.001$). This pattern suggests that the cephalothorax, likely due to the presence of the hepatopancreas and other organs involved in metal accumulation and detoxification, serves as a preferential site for lead bioaccumulation in *L. vannamei*.

Antioxidant response of *S. portulacastrum* to lead exposure

Table 4 presents the levels of protein and non-protein antioxidant compounds in the leaves, stems, and roots of *S. portulacastrum* over eight weeks under Pb exposure and control conditions. Two-way ANOVA showed no significant effects of treatment, time, or their interaction on ascorbic acid (AA) or lycopene (LYP) concentrations. Nonetheless, descriptive trends suggest biological relevance. AA levels were consistently higher in controls, especially during the first two weeks, with values two to three times greater than in Pb-exposed samples, possibly reflecting suppressed synthesis or accumulation under stress. LYP showed a variable pattern: higher in Pb-treated plants in weeks 3 and 7, and in controls during weeks 1 and 2, suggesting non-linear responses possibly linked to temporal or environmental factors.

Table 4

Protein and non-protein antioxidant levels in leaf, stem, and root of *Sesuvium portulacastrum* across weeks in lead-exposed and control groups (mean $\pm$ S.E.; n = 5)

Wk	Peroxidase (U/mg)		Ascorbate peroxidase (U/mg)		Catalase (U/mg)		Ascorbic acid (ug/mg)		Lycopene (mg/mg)	
	Pb-Exposed	Control	Pb-Exposed	Control	Pb-Exposed	Control	Pb-Exposed	Control	Pb-Exposed	Control
1	61.87 $\pm$ 1.08	36.72 $\pm$ 0.72	16.49 $\pm$ 0.41	31.46 $\pm$ 2.89	198.55 $\pm$ 6.67	235.97 $\pm$ 4.31	4.53 $\pm$ 0.30	7.49 $\pm$ 0.70	7.65 $\pm$ 0.56	14.66 $\pm$ 2.89
2	61.53 $\pm$ 7.69	69.59 $\pm$ 9.80	30.95 $\pm$ 1.75	34.45 $\pm$ 0.75	213.05 $\pm$ 7.11	230.04 $\pm$ 9.15	3.74 $\pm$ 0.16	9.97 $\pm$ 1.42	11.81 $\pm$ 3.37	22.21 $\pm$ 3.98
3	91.99 $\pm$ 8.36	38.42 $\pm$ 5.49	17.24 $\pm$ 0.10	20.91 $\pm$ 0.72	148.49 $\pm$ 30.17	129.42 $\pm$ 5.35	3.20 $\pm$ 0.20	4.54 $\pm$ 1.37	23.79 $\pm$ 5.94	13.09 $\pm$ 2.90
4	28.64 $\pm$ 4.09	45.66 $\pm$ 11.42	15.61 $\pm$ 0.27	15.73 $\pm$ 3.22	154.46 $\pm$ 15.34	261.73 $\pm$ 11.67	2.96 $\pm$ 0.28	8.88 $\pm$ 4.64	15.29 $\pm$ 5.89	10.91 $\pm$ 1.96
5	86.55 $\pm$ 6.66	117.19 $\pm$ 9.01	21.84 $\pm$ 0.72	10.45 $\pm$ 1.25	120.24 $\pm$ 32.85	543.72 $\pm$ 7.01	5.14 $\pm$ 1.17	6.45 $\pm$ 2.07	15.31 $\pm$ 3.02	24.14 $\pm$ 7.30
6	55.60 $\pm$ 7.94	76.86 $\pm$ 10.98	15.62 $\pm$ 2.99	18.33 $\pm$ 0.59	98.28 $\pm$ 26.09	694.37 $\pm$ 4.98	2.12 $\pm$ 2.03	3.97 $\pm$ 0.85	17.78 $\pm$ 3.11	24.77 $\pm$ 4.53
7	93.66 $\pm$ 5.51	108.30 $\pm$ 6.37	30.22 $\pm$ 6.98	14.38 $\pm$ 0.14	243.80 $\pm$ 20.76	317.07 $\pm$ 10.42	4.96 $\pm$ 0.28	2.70 $\pm$ 0.69	28.48 $\pm$ 9.49	18.63 $\pm$ 5.00
8	48.21 $\pm$ 9.64	125.62 $\pm$ 11.42	19.14 $\pm$ 0.86	19.75 $\pm$ 0.00	354.72 $\pm$ 16.58	591.87 $\pm$ 29.16	4.54 $\pm$ 0.34	1.97 $\pm$ 0.33	19.32 $\pm$ 4.50	26.13 $\pm$ 5.19

In contrast, protein-based antioxidant enzymes responded strongly to Pb exposure and time. POD activity was significantly affected by treatment ($p = 0.0028$), week ($p < 0.00001$), and their interaction ($p < 0.00001$), with notable increases in Pb-exposed plants during weeks 1 and 3, indicating episodic oxidative defense activation. APX activity was unaffected by treatment ($p = 0.924$) but influenced by time and interaction ($p = 0.034$), suggesting temporally specific responses, particularly in weeks 5 and 7. CAT activity showed the most consistent pattern: treatment, time, and interaction were all highly significant ($p < 0.00001$), with Pb exposure markedly suppressing CAT levels throughout. Differences were especially sharp in weeks 5–8; for instance, in week 6, CAT activity was 98.28 ± 26.09 U/mg in Pb-treated plants vs. 694.37 ± 4.98 U/mg in controls.

Physiological and immunotoxicological response of *L. vannamei* to lead exposure

Lead exposure significantly altered multiple physiological parameters in *L. vannamei*, particularly those related to immune and antioxidant responses (Table 5). Hemocyte count was markedly reduced in Pb-exposed shrimp ($p < 0.000001$), with significant effects of time ($p = 0.00051$) and treatment $\times$ week interaction ($p = 0.023$), suggesting dynamic immunosuppression. NBT reduction, a marker of respiratory burst activity, was also strongly suppressed ($p < 0.000001$), with notable time ($p = 0.00056$) and interaction effects ($p = 0.0072$), indicating oxidative dysfunction.

Table 5

Oxidative, enzymatic, and physiological indicators in the hemolymph of *Litopenaeus vannamei* exposed to lead compared to the control group (mean $\pm$ SE; n = 6)

Wk	Hemocytes (cells/mL $\times 10^6$)		Osmolality (mOsm/L)		NBT reduction rate		Catalase (U/uL)		Lysozyme (U/ μ L)	
	Pb-Exposed	Control	Pb-Exposed	Control	Pb-Exposed	Control	Pb-Exposed	Control	Pb-Exposed	Control
1	7.06 $\pm$ 0.61	6.43 $\pm$ 0.72	1397.70 $\pm$ 49.47	1452.05 $\pm$ 61.31	1.03 $\pm$ 0.04	1.86 $\pm$ 0.08	1.906 $\pm$ 0.16	0.239 $\pm$ 0.07	0.028 $\pm$ 0.01	0.076 $\pm$ 0.01
2	5.47 $\pm$ 1.18	12.12 $\pm$ 1.15	1560.90 $\pm$ 31.95	1275.45 $\pm$ 19.94	0.82 $\pm$ 0.38	1.66 $\pm$ 0.28	1.958 $\pm$ 0.16	0.299 $\pm$ 0.04	0.044 $\pm$ 0.01	0.071 $\pm$ 0.24
3	6.20 $\pm$ 0.6	12.60 $\pm$ 2.56	1484.15 $\pm$ 23.56	1526.30 $\pm$ 80.95	0.95 $\pm$ 0.03	1.67 $\pm$ 0.10	1.753 $\pm$ 0.10	0.555 $\pm$ 0.09	0.038 $\pm$ 0.01	0.121 $\pm$ 0.16
4	4.99 $\pm$ 0.87	8.63 $\pm$ 2.42	1351.20 $\pm$ 32.78	1551.10 $\pm$ 39.20	0.95 $\pm$ 0.06	1.24 $\pm$ 0.27	2.242 $\pm$ 0.15	0.868 $\pm$ 0.06	0.050 $\pm$ 0.01	0.082 $\pm$ 0.19
5	5.61 $\pm$ 1.20	14.50 $\pm$ 1.92	1338.20 $\pm$ 49.09	1456.40 $\pm$ 28.61	0.91 $\pm$ 0.07	1.39 $\pm$ 0.19	2.313 $\pm$ 0.09	0.883 $\pm$ 0.09	0.066 $\pm$ 0.03	0.072 $\pm$ 0.12
6	8.60 $\pm$ 0.66	16.62 $\pm$ 2.40	1274.65 $\pm$ 35.73	1313.90 $\pm$ 31.79	1.04 $\pm$ 0.08	1.46 $\pm$ 0.25	2.097 $\pm$ 0.17	0.775 $\pm$ 0.03	0.107 $\pm$ 0.02	0.084 $\pm$ 0.20
7	7.66 $\pm$ 1.55	12.47 $\pm$ 1.90	1294.30 $\pm$ 11.04	1426.60 $\pm$ 95.36	0.91 $\pm$ 0.09	1.27 $\pm$ 0.07	2.258 $\pm$ 0.17	0.745 $\pm$ 0.50	0.091 $\pm$ 0.01	0.090 $\pm$ 0.19
8	9.33 $\pm$ 0.67	12.46 $\pm$ 1.29	1314.75 $\pm$ 39.96	1248.90 $\pm$ 73.73	0.85 $\pm$ 0.05	1.29 $\pm$ 0.15	1.852 $\pm$ 0.19	0.685 $\pm$ 0.10	0.051 $\pm$ 0.01	0.047 $\pm$ 0.16

Hemolymph osmolality was not significantly affected by treatment ($p = 0.285$), though time ($p = 0.0013$) and its interaction with treatment ($p = 0.0018$) were significant, implying temporal shifts likely related to physiological stress or adaptation. Lysozyme (LYZ) activity was significantly reduced by Pb ($p = 0.009$), with time ($p = 0.031$) and interaction ($p = 0.037$) effects, indicating disruption in innate immune regulation. LYZ values ranged from 0.028 to 0.107 U/ μ L in exposed shrimp, generally lower than in controls (0.047–0.121 U/ μ L), except in week 8. Catalase (CAT) activity was significantly elevated in Pb-treated shrimp ($p < 0.000001$) and varied over time ($p = 0.00001$), though no interaction was observed ($p = 0.278$), suggesting a sustained but parallel temporal pattern across groups.

Biochemical analysis of hemolymph (Supplementary Table S2) showed significantly higher lipid levels in Pb-exposed shrimp (6.7–16.8 mg/mL; $p = 0.0001$), with a time effect ($p = 0.0383$) but no interaction. Carbohydrates varied only with time ($p = 0.0369$), showing minimal differences between treatments. Protein levels were markedly elevated in Pb-exposed individuals (239.9–271.8 mg/mL) compared to controls (88.2–125.6 mg/mL; $p < 0.0001$), with no time or interaction effects, indicating a stable upregulation of protein content under lead exposure.

Microbiota composition in the roots of *S. portulacastrum*

Sequencing and quantification results revealed distinct differences in microbial abundance and DNA yield between control plants and those exposed to lead. Although the total number of sequences retained after size filtering was relatively consistent across all samples (ranging from $\sim 170,000$ to 267,000 reads), the number of unique sequences was markedly higher in Pb-exposed *Sesuvium* roots, with values up to 477, compared to a maximum of 323 in the control group. This suggests a potential increase in microbial diversity under metal-induced stress. In terms of microbial load, Pb-exposed samples exhibited higher gene copy numbers per microliter, with values ranging from 2.6 to 3.7×10^6 genes/ μ L, in contrast to 1.8 to 4.9×10^6 in controls, but with higher consistency and mean values in the contaminated group. The estimated genome equivalents per microliter followed a similar trend, reaching up to $\sim 18,600$ in Pb-treated roots versus a

range of ~ 9,200–24,700 in controls. Although one control sample showed elevated values, Pb-treated samples showed more stable increases across replicates.

Relative composition of bacterial genera in *S. portulacastrum* under lead exposure

The analysis of the bacterial community associated with *S. portulacastrum* roots revealed significant compositional changes in response to lead exposure. As illustrated in Fig. 1a, the relative abundance histograms showed clear differences between the control and Pb-exposed groups. While control samples were primarily dominated by genera such as *Sanguibacter* and *Halomonas*, lead-treated samples exhibited a distinct shift marked by the emergence of genera like *Neptunomonas*, suggesting a reorganization of the rhizosphere microbiota. This alteration likely reflects the selective pressure imposed by heavy metal contamination, which may favor metal-tolerant taxa or disrupt existing microbial interactions.

To further identify taxa most affected by lead exposure, a comparative differential abundance analysis was performed (Fig. 1b). Several bacterial genera exhibited marked changes in their relative abundance between treatments, reinforcing the observation that Pb contamination not only reduces microbial diversity but also alters the dominance patterns within the root microbiome. The consistency of these shifts across replicates supports the reproducibility of the observed effect and highlights potential microbial indicators of environmental metal stress.

The classification analysis using a Random Forest model revealed a clear separation between the root microbiomes of *S. portulacastrum* grown under control conditions and those exposed to lead (Fig. 2). The model, trained on the relative abundance matrix of bacterial genera, achieved a 100% classification accuracy, confirming that lead exposure induces a reproducible and marked shift in microbial community composition. Genera such as *Altererythrobacter*, *Halomonas*, *Blastopirellula*, *Salinigranum*, and *Pleionea* were identified by the model as the most discriminant features, contributing significantly to the classification between treatments. Several of these taxa are typically associated with marine or extreme environments, and belong to families such as Halomonadaceae and Halobacteriaceae, known for their tolerance to salinity and heavy metals. These results suggest that Pb contamination promotes the selection or enrichment of microbial taxa with ecological traits linked to metal resistance or bioremediation, reflecting a functional reorganization of the rhizosphere microbiota in response to environmental stress.

Alpha and beta microbial diversity analysis in the roots of *S. portulacastrum*

Alpha diversity analysis revealed statistically significant differences between control and Pb-exposed (Pb-S) groups. The Simpson evenness index (Simpson-E) was significantly higher in Pb-S samples (Kruskal-Wallis $H = 35.29$, $p < 0.0001$), indicating greater uniformity in the distribution of microbial taxa under lead exposure. Similarly, the Shannon diversity index showed significantly higher values in Pb-S roots compared to controls ($p < 0.0001$), suggesting increased microbial diversity within individual samples (Fig. 3).

Beta diversity analysis based on Bray-Curtis dissimilarity, visualized through multidimensional scaling (MDS), suggested a trend of separation between Pb-exposed and control samples. Nevertheless, this apparent grouping was not statistically supported, as the associated permutation test yielded a p-value of 1.0, indicating no significant difference in overall microbial community composition between treatments.

A Venn diagram comparison revealed 90 genera exclusive to control roots and 104 genera found only in Pb-exposed samples, while 79 genera were shared across both conditions. This partial overlap indicates the presence of a core microbiota, alongside condition-specific taxa potentially associated with stress response or metal tolerance.

Correlations between microbial genera and biochemical variables associated with lead exposure

Significant correlations were detected between the relative abundance of several microbial genera and the biochemical responses of *S. portulacastrum* to lead exposure. Taxa linked to antioxidant activity and enzymatic responses showed strong positive or negative associations with lead levels and oxidative stress markers, suggesting functional relationships between microbiota composition and plant physiological status. Figure 4 presents only statistically significant correlations ($p < 0.05$), visualized as a heatmap indicating both the direction and strength of the associations. Notably, genera affiliated with Halobacteriaceae, Ferrimonadaceae, and *Neptunomonas* were positively correlated with lead concentration and negatively associated with CAT activity, implying a potential role in stress tolerance mechanisms. These findings support the hypothesis that specific microbial groups not only shift in abundance under lead exposure but may also participate in modulating the plant's physiological response.

Discussion

Lead retention in the aquaponic system and its relationship with water nutrient

The phytoremediation efficiency of *S. portulacastrum* in aquaponic systems appears to be modulated by a combination of physiological traits, nutrient dynamics, microbial activity in the biofilter, and overall physicochemical conditions. In several weeks, the plant retained over 90% of dissolved Pb, likely associated with increased metabolic activity and environmental factors that may favor metal uptake. However, retention declined to 36.4% in week 3, which could reflect physiological stress or ionic competition. These fluctuations are in line with reports linking *S. portulacastrum*'s metal tolerance to vacuolar compartmentalization and ion transporters such as NHX3 and SOS1 (Nikalje et al. 2018; Kumawat et al. 2025;). The week-to-week variability supports the idea of a dynamic interplay between biotic and abiotic factors in remediation processes (Mani and Kumar 2014).

Correlations between Pb retention and water chemistry suggest the ionic environment plays a key role. A negative correlation with inlet ammonium ($R = -0.55$) may indicate competitive inhibition between NH_4^+ and Pb^{2+} at root uptake sites, as previously described in agricultural settings (Sun et al. 2023). A similar correlation with total phosphorus ($R = -0.49$) may be related to Pb–phosphate precipitation (Cao et al. 2004). Conversely, a positive correlation with outlet NH_4^+ ($R = 0.42$) could reflect reduced ionic competition or improved uptake conditions (Anjum et al. 2023). Together, these variables explained 55.5% of Pb retention variability in a multiple regression model.

Tissue accumulation of Pb in Sesuvium and shrimp

Both *S. portulacastrum* and *L. vannamei* showed evidence of Pb bioaccumulation, albeit through distinct pathways. In the plant, Pb concentrations were significantly higher in exposed individuals than in controls. While tissue type and exposure time were not significant by ANOVA, weekly patterns suggested internal redistribution. Leaf Pb increased notably in weeks 4, 7, and 8, while stem and root concentrations fluctuated, suggesting possible upward translocation via the xylem. This is consistent with observations by Ghnaya et al. (2013), who proposed the involvement of citric acid in Pb mobility. The stem may function as a transient reservoir, as seen in week 5 ($243.34 \mu\text{g}/100 \text{ g}$), while leaves progressively accumulated Pb, reaching $197.88 \mu\text{g}/100 \text{ g}$ by the end of the experiment. These dynamics align with the root–stem–leaf transfer route described by Jarvis and Leung (2002) and Zaier et al. (2014), potentially mediated by Pb–ligand complexes.

In *L. vannamei*, Pb accumulated preferentially in the cephalothorax, with concentrations consistently higher than in the tail muscle and exceeding $2400 \mu\text{g}/100 \text{ g}$. ANOVA indicated significant effects of treatment, tissue type, and their interaction, but not of time, suggesting that Pb levels may have stabilized after an initial uptake phase. This is compatible with Rainbow's (2002) model describing equilibrium between metal uptake and elimination. The hepatopancreas, located in the cephalothorax, may contribute to this pattern due to its known role in metal detoxification and storage (Osuna-Flores et al. 2014).

Physiological and antioxidant response in *S. portulacastrum* and *L. vannamei*

Lead exposure appears to activate both enzymatic and non-enzymatic antioxidant mechanisms in *S. portulacastrum*. Catalase (CAT) and peroxidase (POD) activities were significantly affected by treatment and exposure time, indicating a temporally regulated response to oxidative stress. These trends are in line with observations by Zhang et al. (2023), who reported increases in CAT and POD under metal stress, with inhibition at high concentrations or prolonged exposure. Ascorbate peroxidase (APX) showed no treatment effect but did respond to time and its interaction with treatment, suggesting involvement in longer-term adaptation, as also proposed by Xiao et al. (2021). Non-enzymatic antioxidants, including ascorbic acid (AA) and lycopene (LYP), did not show statistically significant changes. However, descriptive patterns suggest functional roles: AA levels were higher in controls during early weeks, possibly due to accelerated ROS-driven consumption in exposed plants—a pattern consistent with findings by Bielen et al. (2013). LYP peaks in weeks 3 and 7 in Pb-treated plants may reflect transient oxidative stress events, aligning with reports of carotenoid accumulation under abiotic stress in halophytes (Mansoor et al. 2023).

Subchronic Pb exposure altered several hemolymph parameters in *L. vannamei*. Lipid levels increased significantly over time, possibly reflecting metabolic adjustments to oxidative challenge, as noted in crustaceans under metal stress (Duan et al. 2021; Apun-Molina et al. 2024). In contrast, carbohydrate levels varied with time but not treatment, suggesting they are less sensitive to Pb exposure (Nováková et al. 2015; Zhao et al. 2025). Protein levels were significantly elevated in Pb-treated shrimp and remained stable, possibly indicating ongoing synthesis of detoxification-related proteins such as metallothioneins or heat shock proteins (Wu et al. 2017; Zhao et al. 2025).

Immune and oxidative biomarkers reinforced signs of physiological stress. Pb exposure led to a marked reduction in hemocyte count, and although osmolality remained globally stable, a significant time interaction suggests possible osmoregulatory disruptions. The decline in hemocytes aligns with reports linking Pb to oxidative apoptosis (Frías-Espéricueta et al. 2009). NBT reduction was strongly inhibited and showed treatment–time interaction, suggesting progressive impairment of hemocyte respiratory capacity, similar to patterns observed in cadmium-exposed shrimp (Pourang et al. 2004). Lysozyme (LYZ) activity also decreased significantly under Pb exposure, with relevant time effects, supporting progressive immunosuppression possibly linked to tissue damage in enzyme-producing organs (Duan et al. 2021). In contrast to the plant response, CAT activity increased in shrimp, likely as a compensatory response to H₂O₂ accumulation—a pattern consistent with its protective role in crustaceans under oxidative stress (García-Triana et al. 2010). This complex physiological profile highlights *L. vannamei* hemolymph as a sensitive bioindicator of subchronic lead toxicity.

Rhizosphere microbiota and functional links

Lead exposure in the aquaponic system appeared to restructure the rhizosphere microbiota of *S. portulacastrum*, involving both taxonomic shifts and changes in community diversity. Taxonomic profiling revealed an enrichment of genera typically associated with metal-contaminated or extreme environments, including *Neptunomonas*, *Ferrimonas*, *Arcobacter*, *Navicula*, and *Marinobacterium*. For example, *Neptunomonas* has been isolated from polluted sediments and is capable of hydrocarbon degradation and metal sequestration (Wiratno et al. 2025); *Ferrimonas* is involved in iron reduction under anoxic conditions; *Arcobacter* shows resistance to antimicrobials and heavy metals; and *Navicula* is known for intracellular metal accumulation. The increased abundance of these taxa, coupled with their strong correlation to biochemical markers such as Pb accumulation and CAT activity, and their discriminative power in the Random Forest model, suggests their potential as bioindicators of Pb exposure.

In contrast, groups such as *Cytophagia*, Gemmatimonadetes, and Ectothiorhodospiraceae decreased in relative abundance, suggesting that Pb imposes selective pressure, favoring tolerant taxa while suppressing sensitive ones. This is consistent with contaminant-driven dysbiosis. Interestingly, genera like *Altererythrobacter* and *Halomonas*, although not abundant overall, emerged as strong predictors in the Random Forest model. This highlights the value of supervised

learning in detecting subtle but ecologically relevant microbial shifts that may be overlooked using abundance-based methods. The model classified control and Pb-treated samples with perfect accuracy, reinforcing the robustness of the observed patterns.

Structurally, alpha diversity metrics (Simpson-E and Shannon index) showed a significant increase under Pb exposure. This may reflect the decline of dominant taxa, allowing more stress-tolerant, niche-adapted microbes to colonize. Similar responses have been described in other systems under metal stress, where community restructuring enhances functional traits such as metal sequestration and phosphatase activity (Shade 2023).

Although beta diversity analysis (Bray–Curtis) did not yield statistically significant differences, MDS plots showed visual separation between groups. This inconsistency may stem from the limited sample size ($n = 3$), which is known to reduce statistical power (Eggers et al. 2023). Still, the presence of exclusive genera in each condition, as shown in the Venn diagram, suggests partial community differentiation. This supports the model proposed by Osuna-Flores et al. (2014), in which contamination reshapes community composition by altering genus dominance and evenness, rather than replacing taxa entirely. Thus, while the core microbiota may remain, its structure and functional potential are modified under Pb stress.

Conclusion

This study demonstrates that lead exposure in aquaponic systems generates complex, system-wide effects—ranging from alterations in water chemistry to physiological changes in plants and animals, as well as shifts in microbial dynamics. *S. portulacastrum* proved to be an effective phytoremediator, retaining over 90% of lead, although its efficiency was modulated by nutrient interactions. The plant exhibited a compartmentalized accumulation pattern and a finely tuned antioxidant response, whereas *L. vannamei* responded with generalized enzymatic activation and immune suppression, suggesting contrasting strategies of stress adaptation.

Lead exposure also reshaped the rhizosphere microbiota, increasing community diversity and enriching taxa known for metal tolerance. These microbial shifts correlated with plant biochemical markers and emerged as strong predictors in supervised machine learning models, supporting their potential as functional bioindicators. The integration of taxonomic profiling, predictive modeling, and diversity metrics revealed a dynamic microbial response to Pb stress and underscored the adaptive capacity of the plant-associated microbiome.

Overall, our findings highlight the importance of an integrated ecological approach for understanding and managing metal contamination in productive systems. This knowledge provides a foundation for developing microbiome-based monitoring tools, functional bioinoculants, and resilient aquaponic designs suited for sustainable operation under environmentally adverse conditions.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

MGK: Conceptualization, Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Validation.
MJSS: Writing – review & editing, Validation, Supervision, Investigation, Methodology. JCO: Investigation, Methodology.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

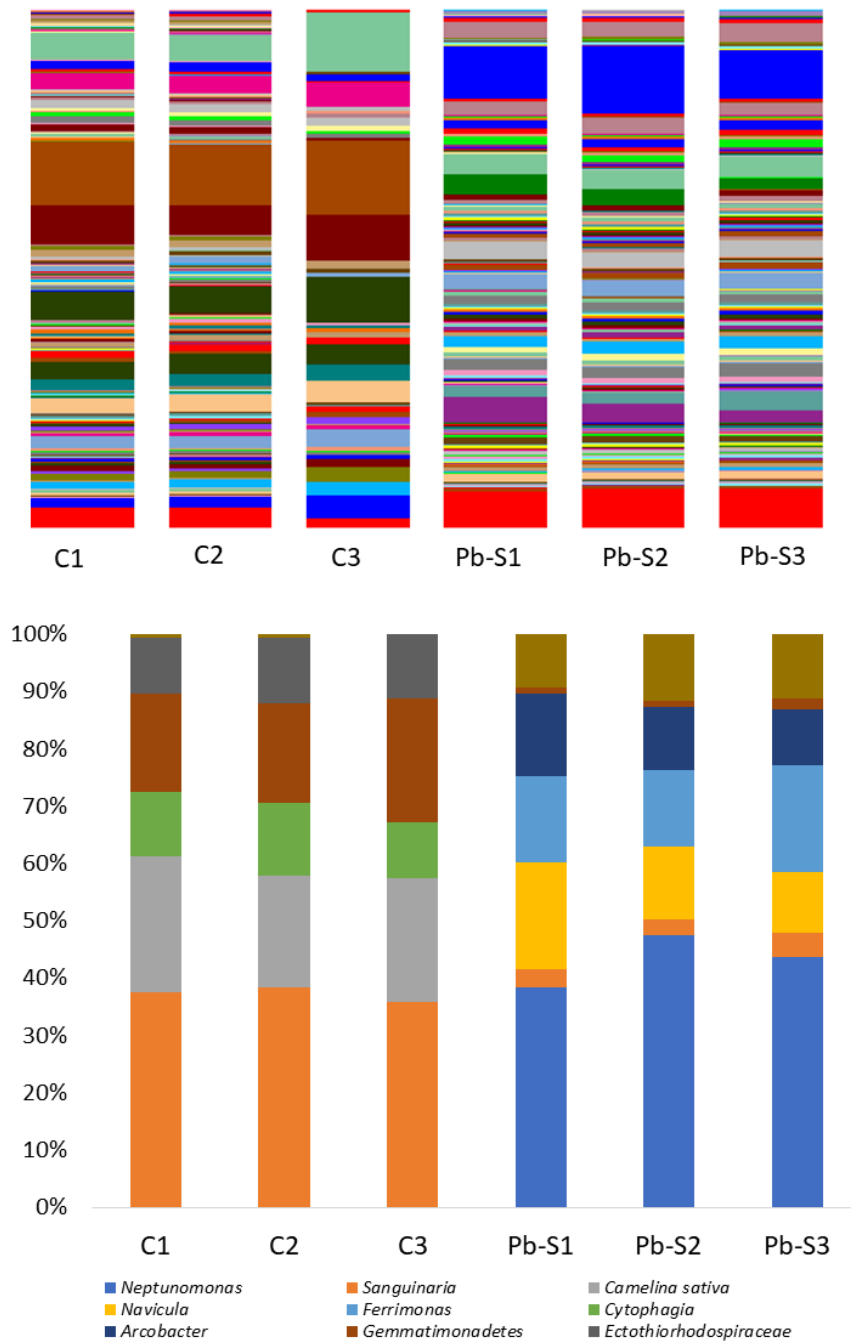


Figure 1

Relative abundance of microbial genera in control (C1–C3) and lead-exposed (Pb-S1–Pb-S3) samples. Top: stacked bar plot showing genus-level diversity across samples. Bottom: dominant genera representing the highest relative abundance in each condition.

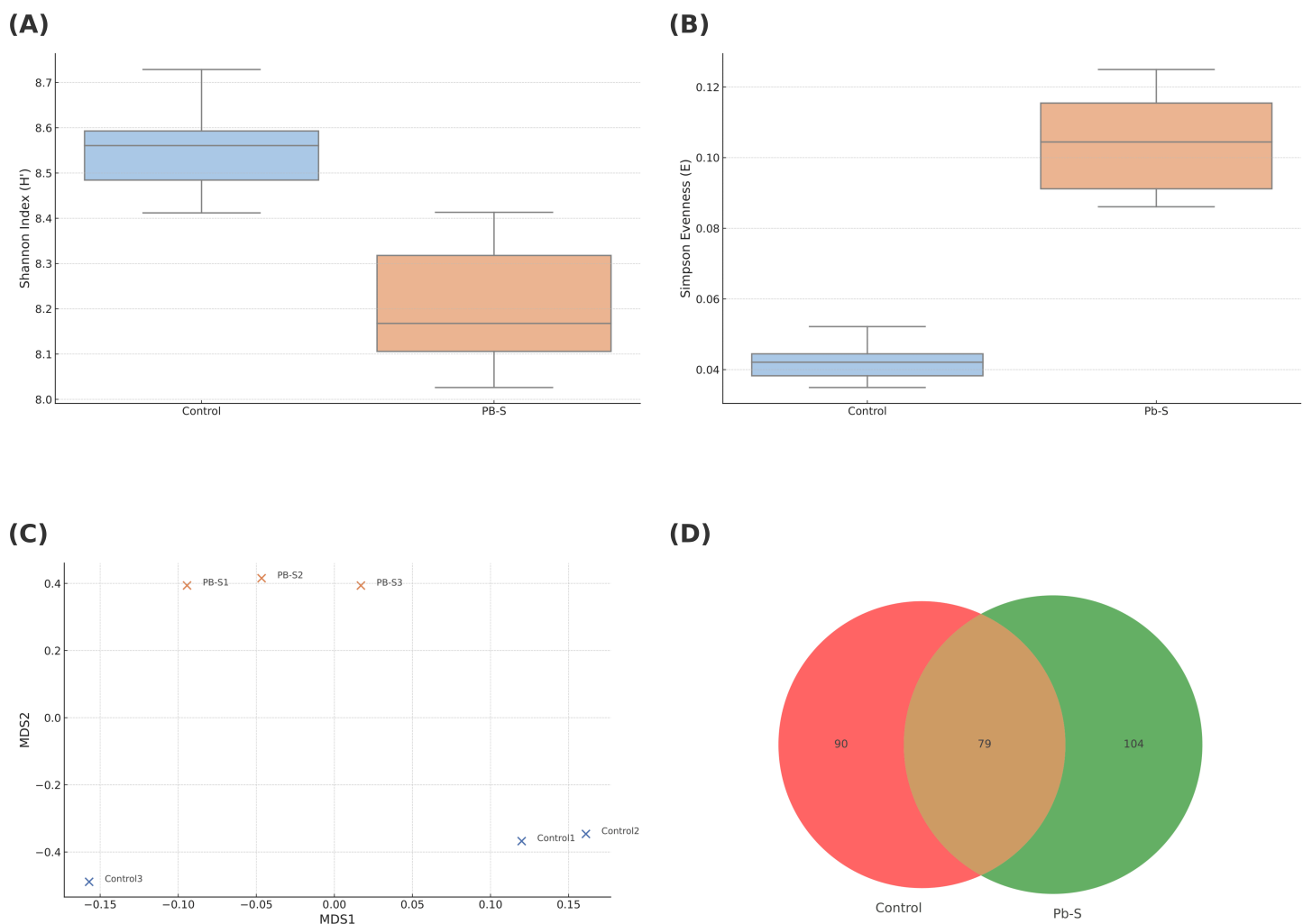


Figure 2

Microbial diversity and composition in control and lead-exposed (Pb-S) samples. (A) Shannon diversity index (H') showing a decrease in overall microbial diversity under Pb exposure. (B) Simpson's evenness (E) indicating increased dominance of specific taxa in Pb-S samples. (C) Multidimensional scaling (MDS) plot based on Bray-Curtis dissimilarity, illustrating clear separation between control and Pb-S groups. (D) Venn diagram displaying the number of unique and shared amplicon sequence variants (ASVs) between control and Pb-S conditions.

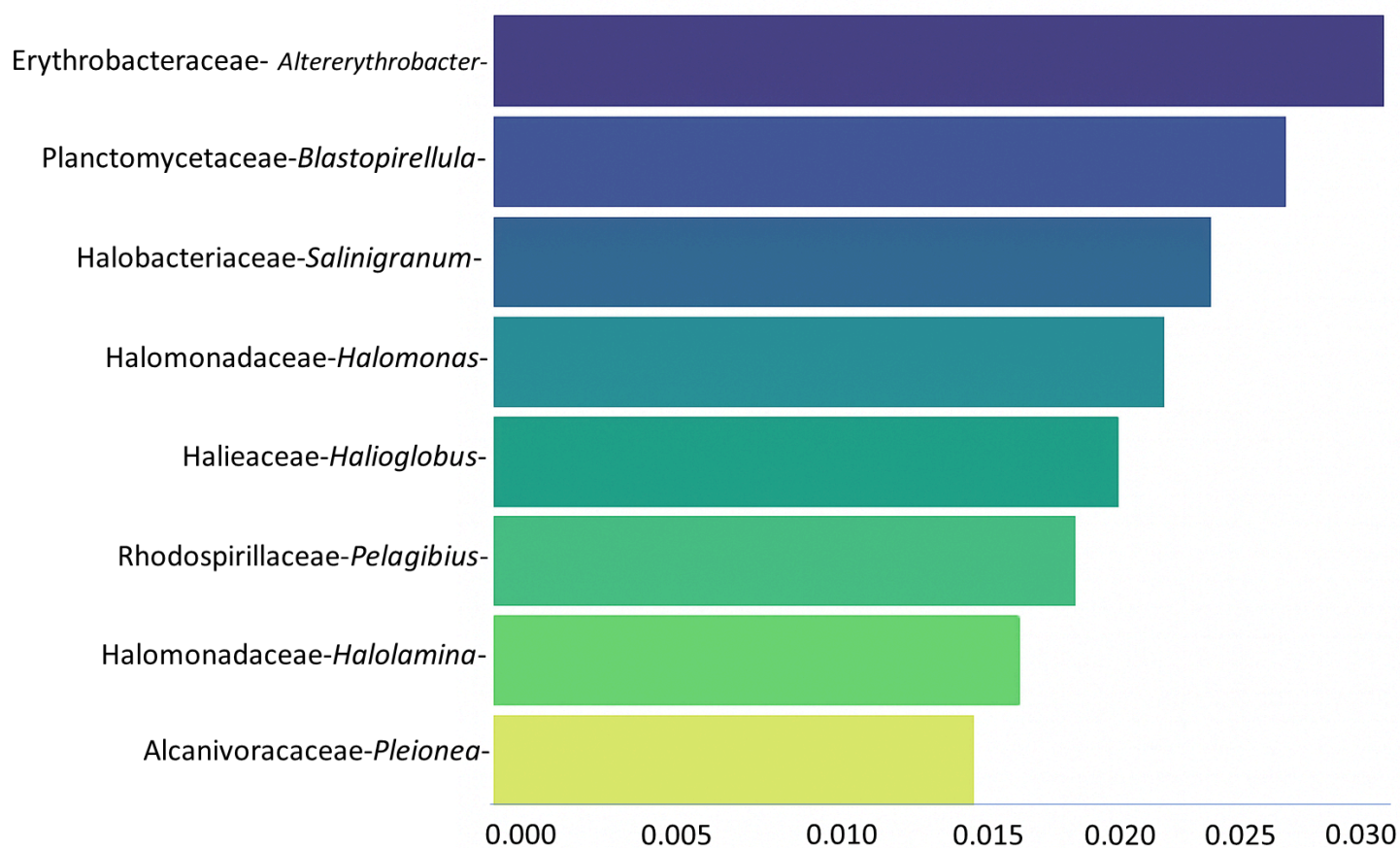


Figure 3

Top microbial genera contributing to the classification of control and lead-exposed conditions using Random Forest analysis. The bar chart presents the ten most important genera ranked by their contribution to reducing classification error. Each bar indicates the relative importance of a genus, with associated taxonomic families included to provide phylogenetic context.

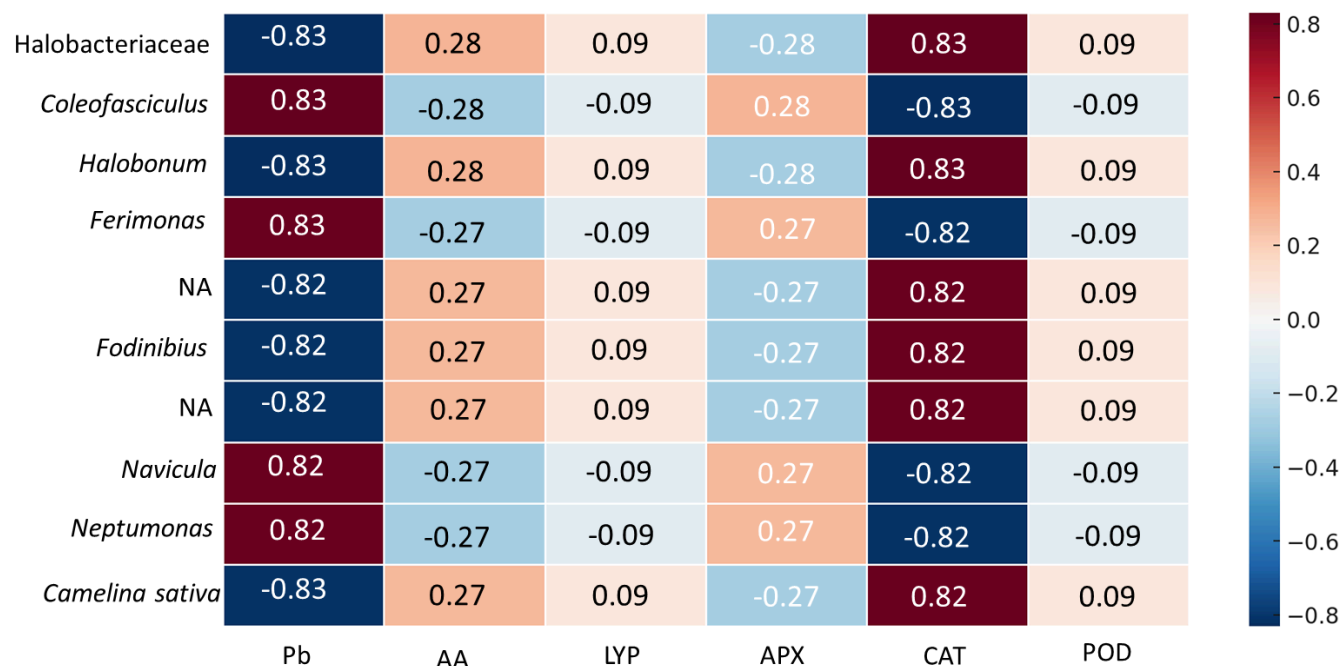


Figure 4

Heatmap of Spearman correlations between the relative abundance of the most variable microbial genera and biochemical variables Measured in the Roots of *Sesuvium portulacastrum*. Only genera with a mean relative abundance > 1% and a standard deviation > 0.5 were included. The heatmap displays only statistically significant correlations ($p < 0.05$), ensuring the inclusion of robust and biologically meaningful associations. Colors indicate the direction and magnitude of the correlation: red represents positive associations, and blue represents negative ones. Pb = Lead; AA = Ascorbic acid; LYP = Lycopene; APX = Ascorbate peroxidase; CAT = Catalase; POD = Peroxidase

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