

Thesis of Doctoral (PhD) Dissertation

**INVESTIGATION OF THE ROLE OF SELENIUM FORMS IN
BIOLOGICAL SYSTEMS (SOIL, PLANTS, AND ANIMALS)**

Arjun Muthu

Dissertation supervisor: Dr Béni Áron



UNIVERSITY OF DEBRECEN

Doctoral School of Food Sciences

Debrecen, 2026

1. THE ANTECEDENTS AND THE OBJECTIVES OF THE DOCTORAL DISSERTATION

Selenium (Se) is an essential trace element required for normal physiological functions in plants, animals, and humans. It plays a critical role in antioxidant defence, thyroid hormone metabolism, immune regulation, and cellular redox homeostasis. Despite its biological importance, selenium is characterised by a narrow margin between nutritional requirement and toxicity. Both selenium deficiency and excess intake are associated with adverse health effects, making accurate assessment, controlled application, and safety evaluation of selenium vital in environmental, food, and feed systems. One of the defining challenges in selenium research is its complex chemical behaviour. Selenium exists in multiple oxidation states and chemical forms, including inorganic species (selenite and selenate), organic forms (selenomethionine and selenocysteine), elemental selenium (Se^0), and nanoscale selenium. These species differ substantially in mobility, bioavailability, toxicity, and metabolic fate. As a result, total selenium concentration alone is often insufficient to describe selenium behaviour or biological relevance. Instead, selenium speciation, transformation pathways, and exposure context must be considered to obtain a meaningful understanding of its environmental and biological impact. The distribution of selenium in soils is highly heterogeneous and controlled by a combination of geological, geochemical, and pedological factors. Large-scale, harmonised datasets are therefore essential for establishing reliable baseline concentrations and identifying regions of potential selenium deficiency or excess. While such datasets provide valuable context, they also highlight a fundamental limitation: bulk soil selenium data cannot resolve selenium speciation, reactivity, or bioavailability, which are critical for assessing environmental risk and nutritional relevance. This limitation creates a direct link between environmental assessment and the need for advanced analytical methods capable of targeting reactive and biologically relevant selenium species. Among selenium intermediates, hydrogen selenide (H_2Se) occupies a central position in selenium biochemistry. H_2Se acts as a key metabolic intermediate linking inorganic selenium compounds to biologically active forms such as selenocysteine. It is also increasingly recognised as a highly reactive redox species involved in cellular signalling and enzymatic regulation. Despite its importance, H_2Se remains poorly characterised due to its extreme chemical instability, volatility, and rapid transformation in aqueous and biological

environments. Conventional selenium speciation techniques are generally optimised for stable selenium compounds and require complex sample preparation steps that preclude direct quantification of free H_2Se . This analytical gap limits the mechanistic understanding of selenium redox cycling and metabolism. Beyond dissolved and volatile selenium species, elemental selenium (Se^0) has gained increasing attention due to its widespread use in nano-enabled food, feed, and biomedical applications. Compared to inorganic selenium salts, Se^0 often exhibits improved bioavailability and reduced toxicity. However, their safety profiles depend strongly on dose, particle properties, exposure duration, and the biological system. Reliable detection of Se^0 in real matrices and systematic evaluation of its biological effects are therefore essential for responsible application. Traditional analytical techniques for elemental selenium determination are often time-consuming, expensive, and laboratory-bound, limiting their suitability for rapid screening and routine monitoring. In this context, fluorescent nanomaterials, particularly carbon nanodots (CNDs), have emerged as promising alternatives for selenium sensing. CNDs exhibit strong and tunable photoluminescence, high chemical stability, water solubility, and excellent biocompatibility. Importantly, they can be synthesised via green, low-cost routes using food-grade precursors, making them suitable for applications in environmental and food-related systems. Surface-functionalised CNDs enable selective interactions with target analytes, allowing the development of simple, rapid, and matrix-tolerant fluorescence-based sensing platforms for elemental selenium and related species. Selenium chemistry also offers unique opportunities for hybrid sensing strategies. The strong chemical affinity between elemental selenium and mercury ions can be exploited to develop responsive sensing systems that transform selenium's reactivity into an analytical advantage. Such approaches are particularly relevant given the global concern surrounding mercury contamination and the need for accessible detection tools. While analytical innovation is essential, understanding the biological implications of selenium exposure remains equally critical. Selenium toxicity is highly dose-dependent and species-specific, and experimental evidence is scattered across diverse biological models with limited standardisation. Conventional toxicological assessment relies heavily on animal studies, which are resource-intensive and constrained by ethical considerations. As a result, there is growing interest in alternative biological models and data-driven approaches that can complement experimental studies. *Drosophila melanogaster* provides scalable, ethically

acceptable platforms for preliminary toxicity screening. At the same time, machine learning offers powerful tools for integrating heterogeneous toxicity data and predicting biological outcomes across species. Against this background, the present doctoral research adopts an integrated, multi-scale approach to selenium assessment. By combining environmental baseline analysis, advanced analytical method development, nano-enabled sensing strategies, biological evaluation, and machine learning-based toxicity prediction, this work aims to provide a coherent framework for understanding selenium behaviour from environmental occurrence to biological impact. Through this integrative strategy, the thesis seeks to contribute both fundamental insight and practical tools for the safer and more informed use of selenium in environmental monitoring, food and feed systems, and nano-enabled applications. To achieve this, the study combined geochemical assessment, analytical method development, nanomaterial-based sensing, *in vivo* experimentation, and machine-learning modelling. The specific objectives of the study were as follows:

- To assess the spatial distribution and variability of total selenium in European and Hungarian agricultural soils.
- To develop and validate a modified hydride-generation atomic fluorescence spectroscopy (HG-AFS) method.
- To develop a CND-based fluorescence sensing platform for the selective detection of elemental selenium (Se^0) and to construct a CND– Se^0 nanohybrid system for selective mercury (Hg^{2+}) detection.
- To compile and harmonise a cross-species *in vivo* nano-selenium toxicity database and to develop and evaluate machine-learning models for predicting nano-selenium toxicity, and
- To experimentally validate the predictive toxicity framework using biological models of *Drosophila Melanogaster*.

Investigation of the role of selenides in biological systems (soil, plants, and animals)

Integrated Assessment of Selenium:
Occurrence → Detection → Sensing → Toxicity → Prediction

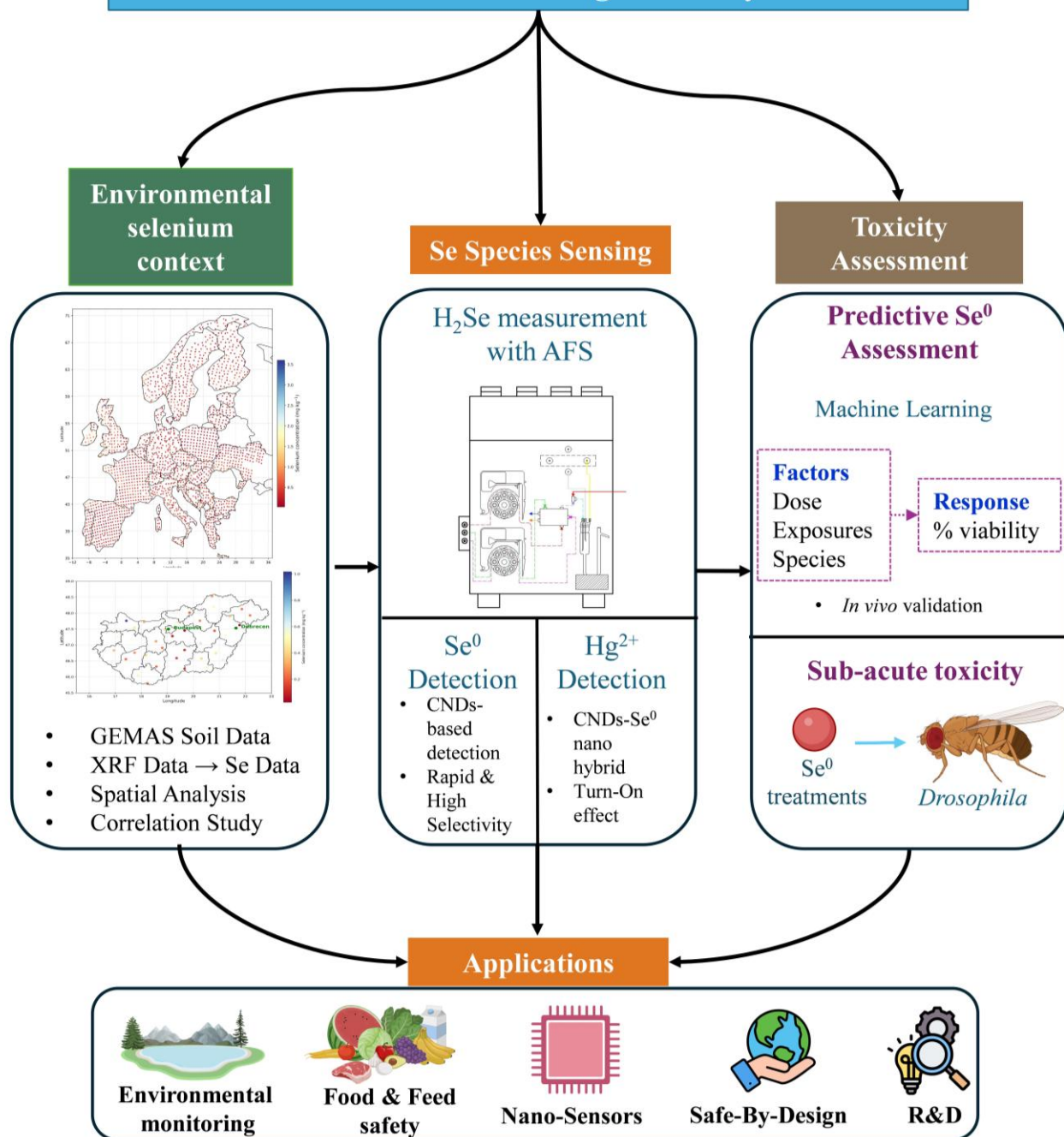


Figure 1. Illustration of the Integrated conceptual framework of the thesis objectives and their applications

2. MATERIALS AND METHODS

2.1 Assessment of Selenium Distribution in Agricultural Soils

2.1.1 Data Source and Study Area

Soil Se (Se) data were obtained from the Geochemical Mapping of Agricultural and Grazing Land Soil (GEMAS) project (O'Connor, 2014a, 2014b), a pan-European geochemical survey coordinated by EuroGeoSurveys and partnering national geological surveys. Se concentrations measured by X-ray fluorescence spectroscopy (XRF) were extracted from the GEMAS database, representing total soil Se. A subset comprising all Hungarian sampling points was also generated to support national-scale comparative analysis with the broader European dataset.

2.1.2 Descriptive Statistical Analysis

Descriptive statistical analysis including Sample number (n), mean, median, minimum and maximum values, standard deviation, interquartile range (IQR), Frequency distributions and Comparative boxplots were constructed to evaluate differences in Se concentration ranges and variability between the European and Hungarian datasets.

2.1.3 Correlation Analysis with Soil Parameters

To explore potential relationships between Se concentration and soil properties, Pearson (linear) and Spearman (rank-based) correlation analyses were conducted separately for the European and Hungarian datasets. Correlation results were summarised using heatmaps.

2.2 Development of an AFS Method for Hydrogen Selenide (H₂Se) Determination

2.2.1 AFS Equipment configurations and their modifications

Millennium Excalibur 10.055 Atomic Fluorescence Spectrometer (PS Analytical, Orpington, UK) was used for this experiment. To improve the stability and transfer efficiency of hydrogen selenide (H₂Se) into the atomizer, the original AFS flow path was reconfigured by installing a low dead-volume Teflon T-connector between the sample outlet and the gas-liquid separator (GLS) inlet.

2.2.2 Method Optimisation

2.2.2.1 Instrumental optimisation

Instrumental parameters governing gas-liquid interaction in the modified manifold were optimised using a full factorial (2^2) experimental design. Two key instrumental variables were examined:

- Hydrogen gas flow rate: 0.5×10^{-6} and $1.0 \times 10^{-6} \text{ m}^3 \text{ min}^{-1}$
- Reagent flow rate: 0.5 and 1.0 mL min^{-1}

2.2.2.2 Optimisation of reagent matrix composition

Following instrumental optimisation, the chemical conditions governing hydride generation were refined to maximise H_2Se formation while avoiding excessive foaming or flow instability. Two reagent-related variables were investigated:

- NaBH_4 concentration: 10%, 40%, and 70% (coded as -1 , 0 , and $+1$)
- NaOH concentration: 2%, 8%, and 14%

2.2.3 Method validation

Calibration of the AFS method was performed using seven Se standard solutions at concentrations of 5, 10, 50, 100, 250, 500, and $1000 \mu\text{g L}^{-1}$. All calibration standards were prepared fresh by diluting the Se(IV) stock solution into the optimized reagent matrix. A calibration curve was plotted. LOD and LOQ were calculated.

2.2.4 Trapping and Recovery of H_2Se in different aqueous matrices

The applicability of the developed method and its tolerance toward matrix effects were assessed using three different aqueous media: ultrapure water, laboratory tap water, and commercial mineral water (ALDI, Debrecen, Hungary). 5, 10, and $100 \mu\text{g L}^{-1}$ concentrations of Se(IV) standards were used for spiking.

2.2.5 Cross-Validation via GF-AAS

Shimadzu AA-7000 (Shimadzu, Japan) Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) was used as an independent validation method for selenium recovery after trapping experiments. 5, and $100 \mu\text{g L}^{-1}$ concentrations of Se(IV) standards were used for spiking and compared with the AFS method.

2.3 Rapid detection of Se⁰ and application to Hg²⁺ detection

2.3.1 Rapid detection of Se⁰

2.3.1.1 Synthesising of Carbon nanodots (CNDs) and Se⁰

CNDs were synthesized using a hydrothermal carbonization approach adapted from a previously reported method (H.H. Nguyen et al., 2024). Se⁰ was prepared following the method of Badgar and Prokisch (Badgar & Prokisch, 2021), where selenite was mixed with ascorbic acid in a 1:10 molar ratio in water. This green reduction reaction yields red Se⁰. All samples were filtered through a 0.22 µm filter before further analysis.

2.3.1.2 CNDs Stability Analysis

To assess the stability of CNDs, fluorescence spectra were collected over a pH range from 3 to 11, for 0 to 60 minutes, and at NaCl concentrations between 0.1 and 1.0 mol L⁻¹. Measurements were taken using an excitation wavelength of 360 nm, an emission range of 300-700 nm, and a scan speed of 1000 nm per minute. All measurements were performed at 25 ± 0.1°C.

2.3.1.3 Selectivity and Validation

To evaluate the selectivity of the synthesized CNDs for Se⁰, interference studies were performed with various potential interfering ions. A concentration of 100 mg L⁻¹ was prepared for each metal ion solution, including Ca²⁺, K⁺, Fe³⁺, Mg²⁺, Mn²⁺, Ni²⁺, Na⁺, and Zn²⁺. Each metal ion was tested both with and without CNDs under identical conditions, and the response was recorded. Se⁰ was detected in ultra-pure water, tap water from a laboratory faucet, and soft drink (7-Up) from a supermarket (Aldi, Hungary) as real-world samples. The samples were analyzed using the spiking method, with added Se⁰ at concentrations of 316.5 µmol L⁻¹ and 633.3 µmol L⁻¹. Three tests were conducted, and the average results were reported.

2.3.2 Application of Selenium-based nanohybrids to detect Hg²⁺

2.3.2.1 Synthesis of Nanohybrids (CND-Se⁰)

10 mg of dry CND powder, dissolved in 10 mL ultrapure water, was combined with 10 mL of 1 mM sodium selenite (Na₂SeO₃). To trigger the reduction of selenite to elemental selenium (Se⁰), 1 mL of 0.1 mM ascorbic acid solution was added dropwise while stirring continuously at room temperature (Badgar & Prokisch, 2021). The CNDs served as both

stabilizers and dispersants, thereby forming a stable CND-Se⁰ nanohybrid suitable for fluorescence analysis.

2.3.2.2 Stability Analysis

The stability of the CND-Se⁰ nanohybrid was evaluated under various environmental conditions, including pH (3–11), incubation time (0–60 minutes), and ionic strength (NaCl concentrations from 0.1 to 1.0 mol L⁻¹).

2.3.2.3 Selectivity and Validation

To assess the selectivity of the synthesized CND-Se⁰ nanohybrid toward Hg²⁺, potential interference from common metal ions was tested. Solutions of 100 mg L⁻¹ for As³⁺, Ca²⁺, Cu²⁺, K⁺, Pb²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Na⁺, Zn²⁺, and Hg²⁺ were prepared. To validate the method on real samples, Hg²⁺ detection was performed in ultrapure water, tap water (from a laboratory faucet), and commercial mineral water (from Aldi, Debrecen, Hungary). The samples were spiked with Hg²⁺ at 1, 3, and 5 µmol L⁻¹, each analyzed in triplicate.

2.4 Data-Driven and Biological Toxicity Assessment of Nano-Selenium

2.4.1 Data Acquisition and Database Construction

A systematic literature search was performed in PubMed to gather *in vivo* nano-selenium toxicity data. The search included terms such as “*nano selenium*”, “*selenium nanoparticles*”, “*toxicity*”, “*LD50*”, “*viability*”, “*in vivo*”, and “*animal model*”.

2.4.2 Exploratory Data Analysis (EDA)

2.4.2.1 Principal Component Analysis (PCA)

PCA was conducted on the standardized continuous variables to reduce dimensionality and identify the most influential predictors of total variance. The PCA was performed using the scikit-learn library, and only the first two principal components were retained to assess variance and visually explore the multivariate structure.

2.4.2.2 Correlation analysis

Pearson correlation coefficients were calculated between all continuous variables to measure linear relationships. These analyses helped identify collinearity, evaluate the relative importance of exposure descriptors, and guide the selection of biologically meaningful predictors for modeling. Based on these exploratory analyses and mechanistic relevance, three features: dose (mg Se kg⁻¹), exposure duration (h), and animal model were chosen for machine-learning model development.

2.4.3 Machine Learning Workflow

2.4.3.1 Dataset split

The dataset was divided into training and testing sets using a 70:30 split, ensuring balanced representation of species across both sets. This division enabled an unbiased assessment of the model's generalization ability.

2.4.3.2 Model selection, Training and evaluation metrics

Model performance was quantified using the Coefficient of determination (R^2), Root Mean Squared Error (RMSE), Mean Absolute Error (MAE), and 10-fold cross-validation ($K = 10$). The models demonstrating the most potent combination of accuracy, stability, and generalization were chosen for detailed optimization and interpretability analysis with SHAP values. The top-performing model was then selected for experimental validation.

2.4.4 Mechanism Analysis

SHAP (SHapley Additive exPlanations) analysis was used to quantify feature contributions and enhance the interpretability of the final machine-learning model.

2.4.5 *In vivo* experimental Validation Using Quails

The *in vivo* feeding experiment with Japanese quail (*Coturnix japonica*) was part of a collaborative study with Ms Aya Ferroudj from the Doctoral School of Animal Science. The aim of this experiment was limited to secondary data analysis, interpretation of biological outcomes (% viability), and integration of the experimental results into the machine-learning-based toxicity prediction framework developed in this thesis.

2.5 *Drosophila melanogaster* toxicity model

The *in vivo* nanotoxicity of Se^0 was evaluated using a *Drosophila melanogaster* digestion-based exposure model. Elemental selenium (Se^0) was supplemented to the cooled medium at five concentrations: 0.1, 0.5, 1, 2, and 4 mg L⁻¹. Media without Se^0 supplementation served as the control. For each concentration, a minimum of five replicate vials were prepared. Embryo development was monitored daily for 20 days. The number of pupae formed and the number of adult flies emerged were recorded at 12-hour intervals and used as quantitative endpoints for toxicity assessment.

2.6 Statistical Analysis

Data preprocessing, statistical evaluation, and graphical visualization were performed using a combination of Python-based tools and dedicated statistical software. Environmental and analytical datasets were processed using Python (version 3.11). Statistical analyses for experimental and analytical validation studies were conducted using GraphPad Prism (version 9.0, GraphPad Software, USA) and IBM SPSS Statistics (version 26).

3. RESULTS

3.1 Selenium Distribution in Agricultural Soils

3.1.1 Se Concentration Levels in European and Hungarian Agricultural Soils

For the European dataset ($n = 2,113$), Se concentrations ranged from 0 to 3.836 mg kg^{-1} , with a mean concentration of 0.405 mg kg^{-1} and a median of 0.354 mg kg^{-1} . The distribution showed moderate heterogeneity and indicates substantial spatial variability at the continental scale. Hungarian agricultural soils ($n = 37$) exhibited Se concentrations ranging from 0.025 to 1.017 mg kg^{-1} , with a mean of 0.355 mg kg^{-1} and a median of 0.327 mg kg^{-1} . Although the mean Se concentration in Hungary was slightly lower than the European average, the similarity of median values suggests comparable baseline Se levels (Zapletalová et al., 2025).

3.1.2 Spatial Distribution of Selenium at European and National Scales

Se concentrations exhibit pronounced spatial heterogeneity, with low to moderate values dominating most agricultural regions (Reimann et al., 2014). Elevated Se concentrations occur periodically and are geographically localized rather than forming continuous regional patterns (Figure 2).

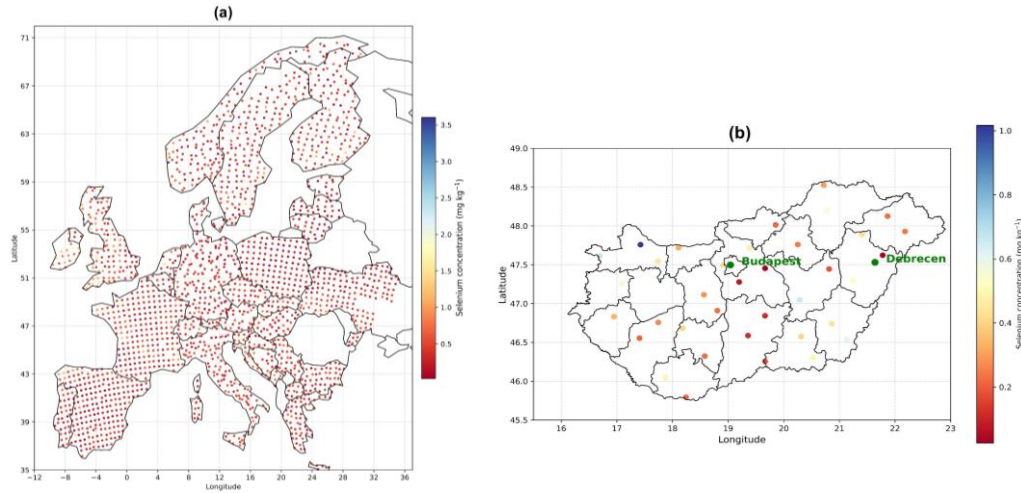


Figure 2. Spatial distribution of Selenium (Se) concentrations in agricultural soils based on GEMAS XRF data:(a) European-scale map; (b) Hungarian-scale map.

3.1.3 Associations Between Selenium and Soil Geochemical Parameters

Overall, the correlation analysis (Figure 3) demonstrates that complex, multivariate geochemical interactions govern Se behavior in agricultural soils. While organic matter and specific elemental associations appear to influence Se distribution, the absence of strong,

universal correlations highlights the limitations of bulk geochemical parameters in predicting Se speciation or bioavailability (Jones & Winkel, 2017).

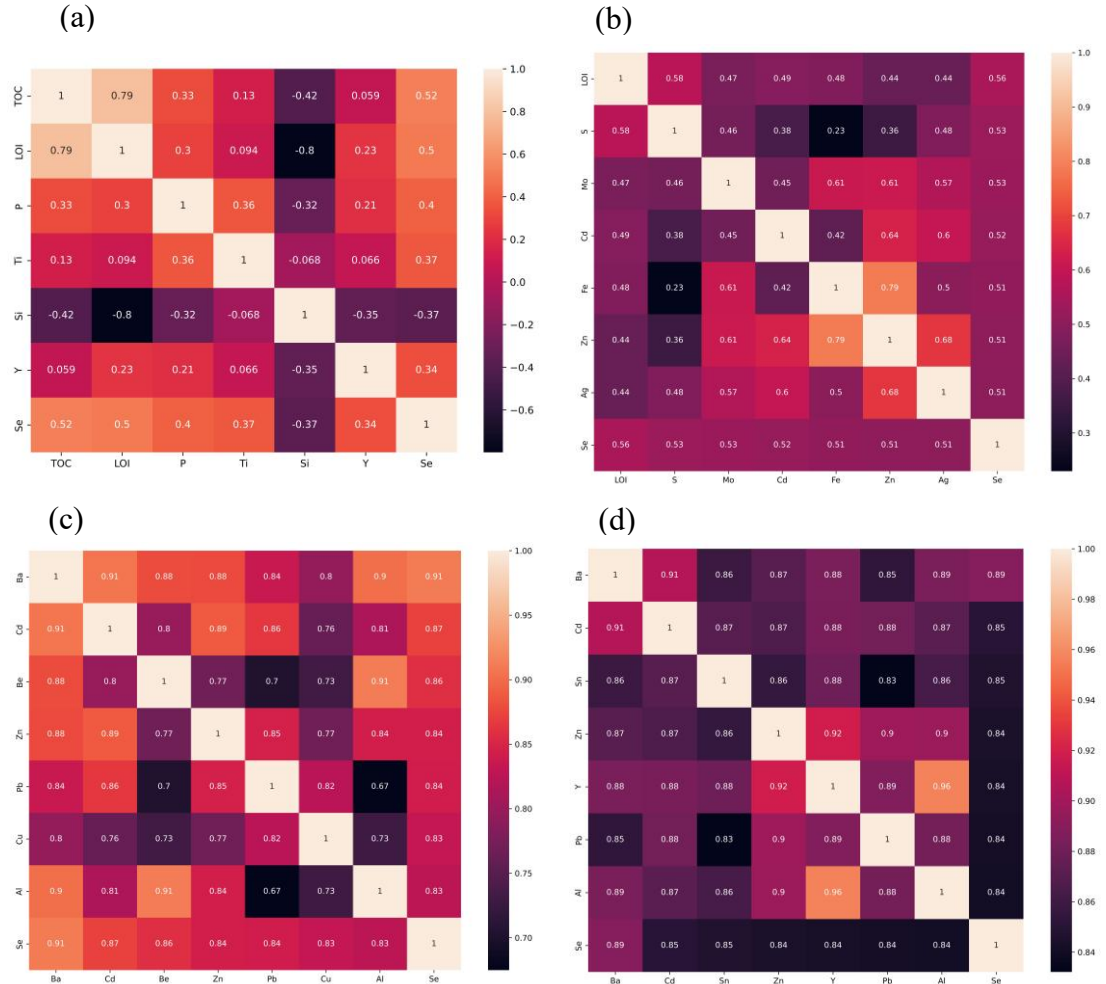


Figure 3. Pearson correlation matrix for Agricultural soil in (a) Europe & (c) Hungary; Spearman correlation matrix for agricultural soils in (b) Europe & (d) Hungary.

3.1.4 Interpretation and Methodological Considerations

The predominance of weak-to-moderate correlations highlights the multifactorial nature of Se behaviour in soils. Se distribution and retention are influenced by a complex combination of factors, including redox conditions, microbial activity, mineralogy, and organic matter composition, many of which cannot be fully captured using bulk geochemical parameters alone. While total selenium concentrations provide essential baseline information, they do not capture selenium speciation or transient intermediates relevant to biological and

environmental transformations. Volatile hydrogen selenide (H_2Se) represents a critical but analytically challenging species in selenium redox cycling. Therefore, the following section focuses on the development and validation of a modified atomic fluorescence spectroscopy (AFS) method for sensitive and reproducible quantification of H_2Se .

3.2 Development of an Atomic Fluorescence Method for Hydrogen Selenide (H_2Se)

3.2.1 Instrumental Optimization for Hydride Generation and AFS Stability

The first stage of method development focused on improving hydride-transfer efficiency and stabilizing atomization in the modified AFS system (Figure 4). An externally supplied hydrogen line was incorporated into the manifold to sustain the AFS flame and promote the controlled reduction of selenium species to H_2Se following in-line acidification of the alkaline sample. A low dead volume Teflon T-joint was positioned between the sample outlet and the gas-liquid separator (GLS) to ensure rapid mixing and minimize hydride decomposition before separation. Only the high-flow combination produced a stable flame, consistent hydride introduction, and reproducible signals.

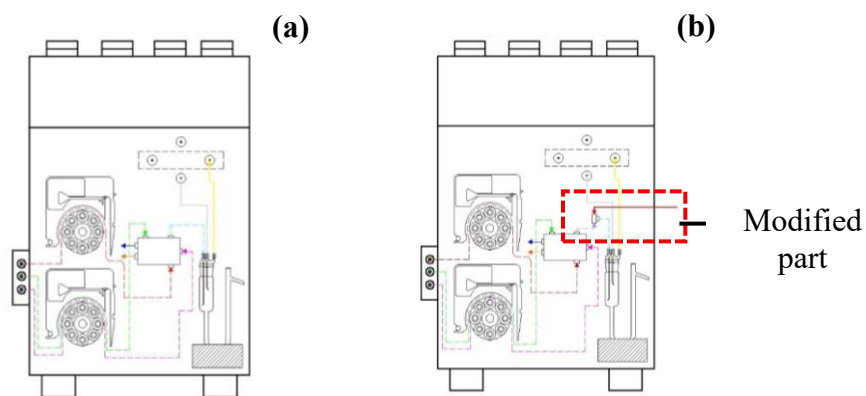


Figure 4. 2D representation of the Atomic Fluorescence Spectroscopy (AFS) Instrument (a) before modification and (b) after modification

3.2.2 Optimization of Reagent Composition

The highest signal intensity (6300.49 mAU) was achieved at 40% NaBH_4 and 8% NaOH , indicating that this combination provides an optimal balance between reductive strength and solution stability.

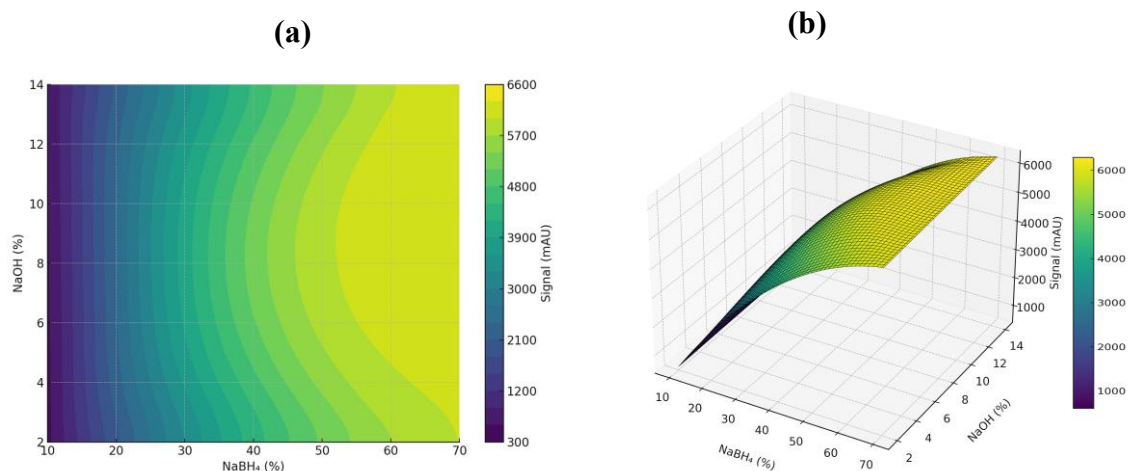


Figure 5. (a) Contour plot and (b) 3D Surface plot

3.2.3 Method Validation

3.2.3.1 Quantitative performance of the optimized method

Fluorescence intensity was plotted against concentration, and the curve (Figure 6) showed excellent linearity across the tested range, with a regression coefficient of determination $R^2 = 0.9973$, with LOD of $1.4 \mu\text{g L}^{-1}$ and LOQ of $4.2 \mu\text{g L}^{-1}$.

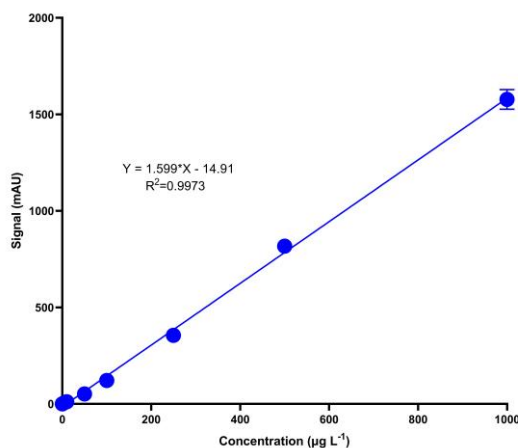


Figure 6. Calibration curve of selenium standards (0–1000 $\mu\text{g L}^{-1}$)

3.2.3.2 Recovery and spiking of H₂Se

The accuracy and trapping efficiency of the developed AFS method were evaluated using spike-and-recovery experiments at two concentration levels (5 and $100 \mu\text{g L}^{-1}$) across three aqueous matrices: ultrapure water, tap water, and commercial mineral water. Across all

matrices and spike levels, recoveries ranged from 97.31% to 101.77%, with RSD values below 3.3%.

3.2.3.3 Cross-validation Using GF-AAS

GF-AAS was used to confirm the total amount of selenium recovered in the NaOH trap after hydride generation. Recovery values of $60.2 \pm 0.34\%$ for $5 \mu\text{g L}^{-1}$ and $100.9 \pm 0.30\%$ for $100 \mu\text{g L}^{-1}$ were obtained, with RSDs of 0.24% and 3.26%, respectively. The lower recovery observed at $5 \mu\text{g L}^{-1}$ reflects the known limitations of GF-AAS for selenium, confirming the modified AFS workflow as a more reliable and operationally efficient platform for trace-level H_2Se analysis, particularly in aqueous matrices where hydride behaviour plays a critical role. (Heilier et al., 2005).

3.3 Carbon Nanodots (CNDs) for Elemental Selenium (Se^0) Detection

3.3.1 Characterisation of Synthesised CNDs

CNDs were characterised by transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), fluorescent spectroscopy, and Raman spectroscopy. The average particle diameter was calculated to be $3.90 \pm 1.36 \text{ nm}$. Highest intensity seen at an excitation wavelength of 360 nm and an emission maximum at around 430 nm. Raman spectroscopy reveals significant peaks: the D band at 1324 cm^{-1} , the F band at 1406 cm^{-1} , and the G band at 1596 cm^{-1} .

3.3.2 Fluorescence stability of CNDs

The fluorescence stability of CNDs is evaluated as a function of pH, salt concentration, and time. Increasing the NaCl concentration resulted in a gradual decrease in CND's fluorescence intensity. There is no significant fluorescence shift over 60 min, and CNDs emitted strong fluorescence across 3 and 11. At neutral pH (7), the lowest fluorescence was recorded, indicating a substantial quenching effect.

3.3.3 Calibration, Sensitivity, and Selectivity towards Se^0

Over a ten-point calibration range from 0 to $12.665 \text{ mmol L}^{-1}$, the linear calibration line (Figure 7a) for Se^0 was established, with an LOD of $0.131 \text{ mmol L}^{-1}$ and an LOQ of $0.397 \text{ mmol L}^{-1}$. Lowest I/I_0 ratio (~ 0.34), indicating strong quenching and the high selectivity of CNDs for Se^0 over the other common metal ions tested (Figure 7b).

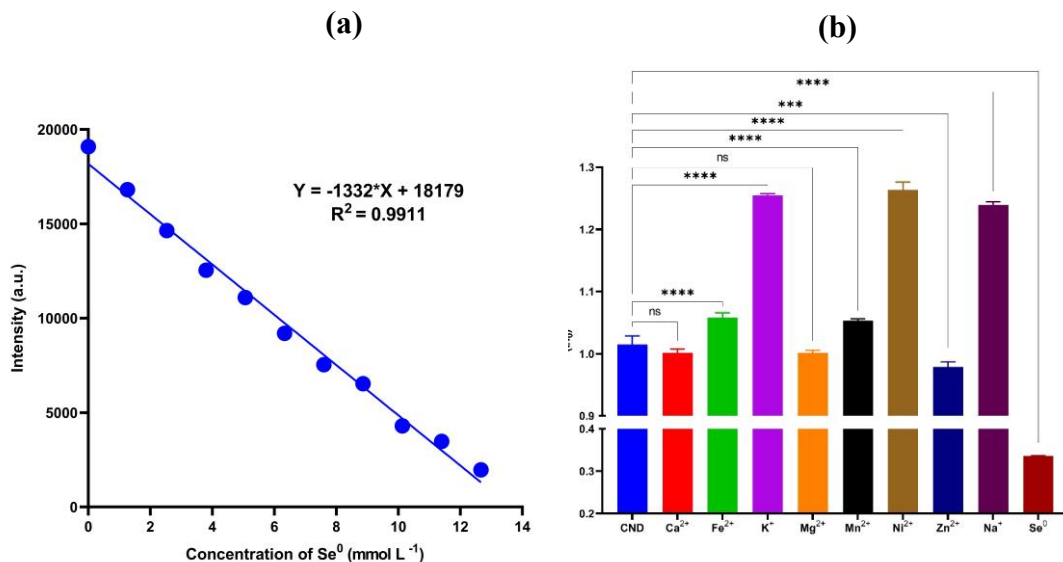


Figure 7. (a) Standard linear calibration curve of Se⁰ and **(b)** Selectivity of CNDs with common metal ions and Se⁰

3.3.4 Application to Real Water and Beverage Matrices

To assess the practical applicability of the developed CND-based fluorescent sensor, spiking and recovery experiments were conducted using ultra-pure water, tap water, and a commercial soft drink matrix. Se⁰ was added at two concentrations (316.5 $\mu\text{mol L}^{-1}$ and 633.3 $\mu\text{mol L}^{-1}$). CNDs have demonstrated excellent recovery ranging from 98.60% to 108.1%, with low RSD values (0.2–3.4%) indicating high precision and reproducibility.

3.4 CND-Se⁰ Nanohybrid System for Mercury (Hg²⁺) Detection

3.4.1 Characterization of CND–Se⁰ Nanohybrids

The particles appeared as uniformly dispersed, dark, spherical structures with an average diameter of 3.12 ± 1.04 nm. The hybrid displayed strong excitation-dependent fluorescence with a maximum emission at 430 nm upon excitation at 360 nm. Raman spectroscopy shows a distinct peak at 251 cm^{-1} , which indicates the A1 vibrational mode of amorphous Se. Additionally, the hybrid retained characteristic carbon peaks, including the D band at 1324 cm^{-1} and the G band at 1562 cm^{-1} .

3.4.2 Stability of the Nanohybrid System

The noticeable enhancement in fluorescence intensity was observed at higher salt concentrations, particularly at 1.0 mol L^{-1} . The fluorescence spectra remained nearly

unchanged during the entire period. Maximum fluorescence emission was observed at acidic pH values (3-4), followed by a sharp decrease from pH 6 to 8. Beyond pH 9, a partial recovery in intensity was observed.

3.4.3 Calibration, Sensitivity, and Selectivity of nanohybrid towards Hg^{2+}

The signal increased proportionally with rising Hg^{2+} concentrations ranging from 0.5 to 15 $\mu\text{mol L}^{-1}$ (Figure 8a). The resulting calibration plot showed excellent linearity with the LOD of 8.7 nmol L^{-1} and LOQ of 26.4 nmol L^{-1} . Highest I/I_0 ratio (Figure 8b) was observed with Hg^{2+} ions, showing the better selectivity of nanohybrids over the other common metal ions.

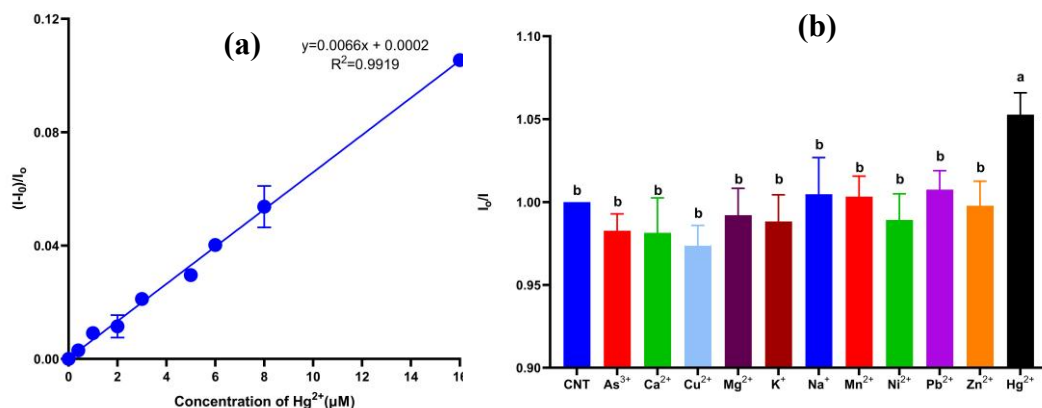


Figure 8. (a) Standard linear calibration curve of Hg^{2+} and (b) Selectivity of nanohybrids with common metal ions and Hg^{2+} .

3.4.4 Real sample validation

The CND- Se^0 fluorescent probe, recovery experiments were conducted using three real water matrices: mineral water, tap water, and ultra-pure water. Each sample was spiked with Hg^{2+} at concentrations of 1.0, 3.0, and 5.0 $\mu\text{mol L}^{-1}$. The results revealed satisfactory recoveries in the range of 99.92–105.2%, indicating the method's accuracy across different sample matrices. RSD values were consistently low ($\leq 3.3\%$), demonstrating good repeatability and precision.

3.5 Machine Learning Modelling of Nano-Selenium Toxicity

3.5.1 Dataset composition and variability

The curated dataset comprised 86 *in vivo* nano-selenium toxicity entries extracted from peer-reviewed studies, representing eight biological models: mice, rats, quail, chicken, fish,

Drosophila, *Lucilia sericata*, and *Spodoptera* larvae. Nano-selenium doses ranged from 0.0375 to 198 mg kg⁻¹.

3.5.2 Exploratory Data Analysis and Feature Selection

3.5.2.1 Principal Component Analysis (PCA) and correlation analysis

PCA was conducted to visualise the multivariate structure and assess whether the predictors produced species-level clustering (Jha et al., 2018). The first two principal components explained approximately 50% of the total variance. PC1 was dominated by dose and exposure duration, whereas PC2 captured inter-species variability (Figure 9a). Pearson correlation coefficients (Figure 9b) were calculated to assess linear relationships among the continuous variables. Dose and viability exhibited a moderate negative correlation, reflecting reduced survival at higher concentrations.



Figure 9. (a) Principal Component Analysis (PCA) of the Nano-Selenium Toxicity Dataset, and **(b)** Pearson Correlation Matrix of Experimental Variables.

3.5.2.2 Machine Learning: Model Screening and Comparative Performance Analysis

Learning strategies were trained on the curated dataset using a consistent 70:30 train-test. AdaBoost was selected as the final model. It achieved $R^2 = 0.971$ (train) and 0.848 (test) with low RMSE and MAE values, effectively capturing the nonlinear relationships among dose, exposure duration, and species. This model was therefore chosen for subsequent SHAP-based interpretability analysis and experimental validation in Japanese quails.

3.5.2.3 Selection and Validation of the AdaBoost Regressor Model

The cross-validated mean R^2 was 0.828 ± 0.099 with a corresponding RMSE of 9.920 ± 4.127 and MAE of 5.724 ± 2.523 . The relatively narrow standard deviations across folds indicate

stable predictive behaviour and minimal sensitivity to data partitioning, supporting the conclusion that the model did not overfit to specific subsets of the dataset (Merabet et al., 2025).

3.5.2.4 Model Robustness: Residual Analysis and Error Distribution

Residual analysis was performed further to evaluate model reliability and bias. Most residuals were tightly clustered around zero, indicating good agreement between predicted and observed viability values across most experimental entries.

3.5.2.5 SHAP Analysis and Leave-One-Out Cross-Validation (LOOCV)

SHAP beeswarm plots (Figure 10a) further illustrated these trends, with higher doses associated with strongly negative SHAP values (reduced predicted viability). Residual distributions (Figure 10b) derived from SHAP analysis were centred near zero, indicating the absence of systematic prediction bias. Global SHAP importance rankings (Figure 10c) identified dose (mg Se kg^{-1}) as the dominant predictor of viability, contributing substantially more to model output than any other variable. RMSE distribution (Figure 10d) showed that most samples exhibited low prediction errors, typically within $\pm 10\%$ viability deviation. Collectively, SHAP and LOOCV analyses demonstrate that the AdaBoost model combines strong interpretability with robust generalisation.

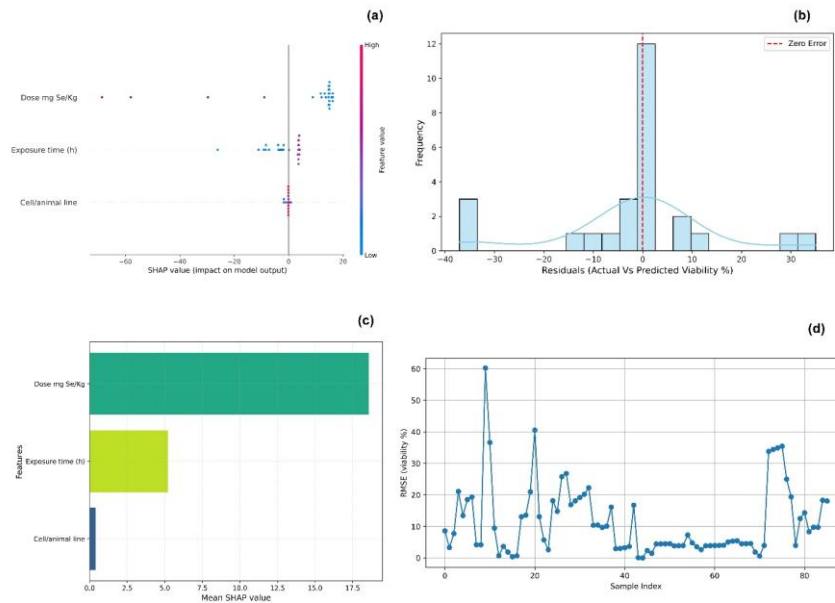


Figure 10. Model explainability and robustness assessment for the AdaBoost regressor. **(a)** SHAP beeswarm, **(b)** Residual distribution, **(c)** Global SHAP importance ranking, and **(d)** LOOCV plot.

3.5.3 Model experimental validation using Quails

3.5.3.1 Characterisation of Selenium Nanoparticles (SeNPs)

The mean particle diameter was 20.05 ± 3.7 nm, a size range associated with enhanced bioavailability while remaining below typical agglomeration thresholds. EDX spectra showed distinct selenium peaks without detectable impurities, confirming chemical purity.

3.5.3.2 *In Vivo* validation using Japanese Quails

Quails were fed diets supplemented with SeNPs at 0.05 and 0.5 mg Se kg⁻¹ feed for 28 days. Viability, defined as survival of clinically normal birds, remained 100% in both treatment groups, consistent with the reported low toxicity of SeNPs within their physiological tolerance range (Khalid et al., 2025).

3.5.3.3 Comparison Between Experimental and Predicted Viability

AdaBoost model predicted viabilities of 99.02% and 99.01% for the 0.05 and 0.5 mg Se kg⁻¹ treatments, respectively. The deviation between predicted and observed outcomes was less than 1%, corresponding to an overall prediction error of 0.988%.

3.5.3.4 Toxicological Significance and Model Reliability

These findings highlight the potential of machine-learning-assisted approaches to support “Safe by Design” nanoparticle implementation in animal nutrition and food systems, in line with emerging regulatory expectations (Zhang et al., 2025; Zouraris et al., 2025). To further resolve dose-dependent developmental effects and capture sub-lethal toxicity mechanisms, *Drosophila melanogaster* was employed as a complementary *in vivo* digestion-based model.

3.6 *In Vivo* Assessment of Elemental Selenium Toxicity in *Drosophila melanogaster*

3.6.1 Pupal Viability and Developmental Kinetics

Survival curves (Figure 11a) demonstrated that pupation onset was delayed at higher Se⁰ concentrations, particularly at 2 and 4 mg kg⁻¹, indicating impaired developmental kinetics. This delay may result from the oxidative stress accumulation, disrupting energy balance and developmental timing (Mishra & Panda, 2021). Bar plot analysis (Figure 11b) further confirmed that pupal viability was significantly enhanced at 1 mg kg⁻¹ compared to the control.

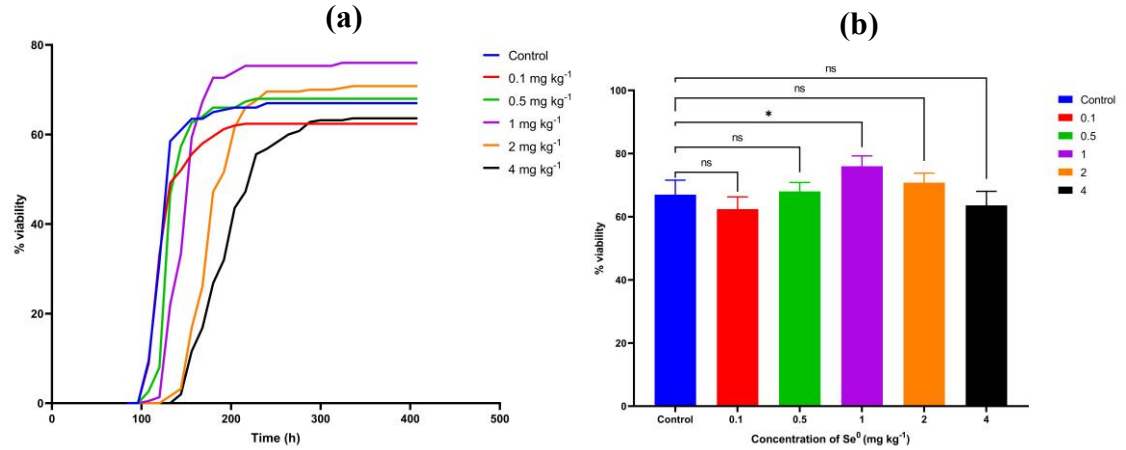


Figure 11. (a) Time-resolved survivability curve of pupae, and **(b)** Viability plot of pupae.

3.6.2 Adult Fly Viability and Survival Outcomes

The time-resolved viability curves indicate that high Se^0 concentrations primarily affected early adult survival rather than causing immediate lethality, suggesting cumulative or delayed toxicity effects. At 1 mg kg^{-1} , adult viability was significantly higher than the control, suggesting that moderate Se^0 exposure during development may confer a survival advantage extending into adulthood (Miksane et al., 2024).

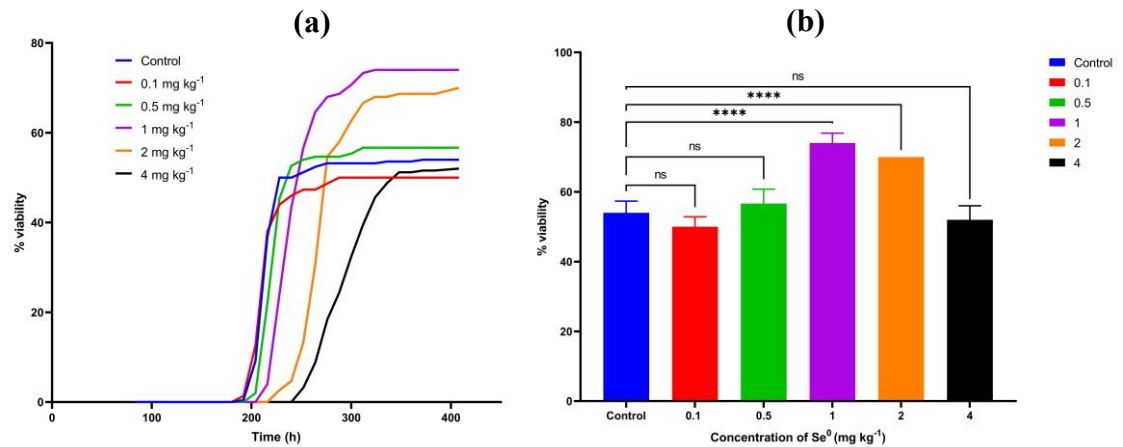


Figure 12.(a) Time-resolved survivability curve of adult fly, and **(b)** Viability plot of adult fly.

3.6.3 Toxicological Interpretation and Hormetic Behaviour

Overall, the results demonstrate a narrow but clearly defined threshold between beneficial and adverse Se⁰ exposure. Low to moderate concentrations support normal development and, in some cases, enhance survival, while higher doses induce developmental stress and reduced viability.

4. NEW CONTRIBUTIONS TO ACADEMIC KNOWLEDGE

The outcomes of this doctoral research provide practical results applicable to environmental monitoring, analytical laboratories, food and feed safety evaluation, and nano-enabled risk assessment. The developed methods and frameworks are scalable, cost-effective, and compatible with real-world matrices.

1. Using harmonized GEMAS XRF data, total selenium concentrations in agricultural soils were systematically evaluated at continental and national scales. For European agricultural soils ($n=2113$), the mean selenium concentration was 0.405 mg kg^{-1} , with a median of 0.354 mg kg^{-1} . Spatial variability was moderate, indicating heterogeneous but stable background levels. For Hungarian agricultural soils ($n=37$), the mean selenium concentration was 0.355 mg kg^{-1} , with a median of 0.327 mg kg^{-1} . This indicates consistent internal variability with continental trends, despite the smaller dataset.
2. A novel, externally assisted hydride-generation atomic fluorescence spectroscopy (HG-AFS) system was developed and validated for the direct quantification of volatile hydrogen selenide (H_2Se). The method exhibited excellent linearity over $5\text{--}1000 \text{ }\mu\text{g L}^{-1}$ with $R^2 = 0.9973$. The limit of detection (LOD) was $1.40 \text{ }\mu\text{g L}^{-1}$, and the limit of quantification (LOQ) was $4.20 \text{ }\mu\text{g L}^{-1}$, with repeatability $<5\%$ RSD. Spike-recovery experiments in ultrapure, tap, and mineral water yielded recoveries of $97.3\text{--}101.8\%$ with $\text{RSD} < 3.3\%$, confirming matrix tolerance. Cross-validation using GF-AAS confirmed low quantitative recovery at $5 \text{ }\mu\text{g L}^{-1}$ concentrations while highlighting the superior sensitivity of the developed AFS method at ultra-trace levels.
3. A rapid method was developed with a carbon nanodot (CND) fluorescence platform for the selective detection of elemental selenium (Se^0) and application in aqueous matrices. A linear calibration was achieved over $0\text{--}12.665 \text{ mmol L}^{-1}$ of Se^0 with $R^2 = 0.9911$; The method sensitivity was quantified as $\text{LOD} = 0.131 \text{ mmol L}^{-1}$ and $\text{LOQ} = 0.397 \text{ mmol L}^{-1}$. Spiking and recovery in ultrapure water, tap water, and soft drink matrices at 316.5 and $633.3 \text{ }\mu\text{mol L}^{-1}$ yielded recoveries of $98.60\text{--}108.1\%$ with $\text{RSD} 0.2\text{--}3.4\%$, confirming robustness in real matrices.

4. A CND-Se⁰ nanohybrid probe was developed with a “turn-on” response to Hg²⁺. A linear calibration was achieved over 0.5-15 $\mu\text{mol L}^{-1}$ of Hg²⁺ with $R^2 = 0.9919$. The method achieved LOD = 8.7 nmol L^{-1} and LOQ = 26.4 nmol L^{-1} , enabling trace-level detection in drinking/environmental water contexts. Spiking and recovery validation in mineral, tap, and ultrapure water at 1, 3, and 5 $\mu\text{mol L}^{-1}$ achieved recoveries of 99.92–105.2% with $\text{RSD} \leq 3.3\%$, confirming accuracy and repeatability across ionic-rich and low-conductivity matrices.
5. A cross-species ML framework was developed to predict viability outcomes from nano-selenium exposure. A curated literature dataset of 86 in vivo entries spanning 8 biological models was compiled. After screening of 13 different models, AdaBoost Regressor had the most balanced performance and interpretability with: Train: $R^2 = 0.971$, Test: $R^2 = 0.848$, and validated with a k-fold validation method. Pearson analysis showed a moderate negative association between dose and viability, while particle size exhibited no meaningful correlation. External validation with quails at 0.05 and 0.5 mg Se kg^{-1} feed yielded observed viability of 100% in both groups, while AdaBoost predicted 99.02% and 99.01%, respectively.
6. A digestion-based *Drosophila melanogaster* model was applied to experimentally quantify developmental and survival responses to elemental selenium (Se⁰), providing stage-resolved in vivo toxicity thresholds relevant for nano-selenium risk assessment. At low-dose Se⁰ (0.1-0.5 mg kg^{-1}), there was no impairment of developmental progression. At moderate Se⁰ exposure (1 mg kg^{-1}), a reproducible hormetic response was induced. At higher Se⁰ doses (2-4 mg kg^{-1}), impaired developmental kinetics and reduced survival.

Together, these results establish an integrated experimental and computational framework that links selenium occurrence, analytical accessibility, nano-enabled sensing, biological responses, and toxicity prediction.

5. PRACTICAL USE OF THE RESULTS

The outcomes of this doctoral research provide several practical results that can be directly applied in environmental monitoring, analytical laboratories, food and feed safety evaluation, and nano-enabled risk assessment. The developed methods and frameworks are scalable, cost-effective, and compatible with real-world matrices.

The harmonised interpretation of GEMAS soil data provides a reliable baseline for evaluating selenium levels in European and Hungarian agricultural soils. This enables identification of selenium-deficient or marginal regions without reliance on speculative bioavailability indices. The modified atomic fluorescence spectroscopy (AFS) method developed for the determination of hydrogen selenide (H_2Se) provides a robust and accessible analytical tool for laboratories lacking advanced mass spectrometric instrumentation. The method is suitable for routine analysis of selenium transformation processes, validation of selenium trapping experiments, and laboratory-scale metabolic studies.

The carbon nanodot (CND)-based fluorescence platforms developed in this work enable rapid detection of elemental selenium and mercury ions without complex sample preparation. These features make the sensing systems suitable for preliminary screening, quality control, and fluorescence-based detection.

The machine-learning toxicity framework provides a practical decision-support tool for evaluating the safety of nano-selenium across species. The successful validation using Japanese quails confirms its utility for guiding experimental design and comparative risk assessment. The digestion-based *Drosophila melanogaster* model established in this study offers a rapid and ethically acceptable platform for preliminary nano-selenium toxicity screening.

Collectively, the practical outcomes of this doctoral research provide integrated tools for selenium assessment that span environmental evaluation, analytical measurement, nanosensing, toxicity prediction, and biological screening.

6. BIBLIOGRAPHY

- Badgar, K., & Prokisch, J. (2021). Simple method for preparing elemental selenium nano-coating inside a silicone surface. *Acta Agraria Debreceniensis*, 1, 35–43. <https://doi.org/10.34101/actaagrar/1/8940>
- Heilier, J.-F., Buchet, J.-P., Haufroid, V., & Lison, D. (2005). Comparison of atomic absorption and fluorescence spectroscopic methods for the routine determination of urinary arsenic. *International Archives of Occupational and Environmental Health*, 78(1), 51–59. <https://doi.org/10.1007/s00420-004-0562-x>
- H.H. Nguyen, D., Muthu, A., El-Ramady, H., Daróczy, L., Nagy, L., Kéki, S., Béni, Á., Csarnovics, I., & Prokisch, J. (2024). Optimization of extraction conditions to synthesize green carbon nanodots using the Maillard reaction. *Materials Advances*, 5(8), 3499–3505. <https://doi.org/10.1039/D4MA00037D>
- Jha, S. Kr., Yoon, T. H., & Pan, Z. (2018). Multivariate statistical analysis for selecting optimal descriptors in the toxicity modeling of nanomaterials. *Computers in Biology and Medicine*, 99, 161–172. <https://doi.org/10.1016/j.compbiomed.2018.06.012>
- Jones, G. D., & Winkel, L. H. E. (2017). Multi-scale Factors and Processes Controlling Selenium Distributions in Soils. In E. A. H. Pilon-Smits, L. H. E. Winkel, & Z.-Q. Lin (Eds.), *Selenium in plants: Molecular, Physiological, Ecological and Evolutionary Aspects* (pp. 3–20). Springer International Publishing. https://doi.org/10.1007/978-3-319-56249-0_1
- Khalid, A., Satti, S., Ayyub, A., Nawab, F., Tahir, M., Naz, S., Almutairi, M. H., Alrefaei, A. F., Khan, R. U., & Ibiwoye, D. I. (2025). Blood, feathers, and eggs as bioindicators of selenium sources and their impact on DNA damage in Japanese quails (*Coturnix japonica*). *Research in Veterinary Science*, 185, 105556. <https://doi.org/10.1016/j.rvsc.2025.105556>
- Merabet, K., Di Nunno, F., Granata, F., Kim, S., Adnan, R. M., Heddami, S., Kisi, O., & Zounemat-Kermani, M. (2025). Predicting water quality variables using gradient boosting machine: Global versus local explainability using SHapley Additive Explanations (SHAP). *Earth Science Informatics*, 18(3), 298. <https://doi.org/10.1007/s12145-025-01796-y>
- Miksanek, J. R., Adarkwah, C., & Tuda, M. (2024). Low concentrations of selenium nanoparticles enhance the performance of a generalist parasitoid and its host, with no net effect on host suppression. *Pest Management Science*, 80(4), 1812–1820.
- Mishra, M., & Panda, M. (2021). Reactive oxygen species: The root cause of nanoparticle-induced toxicity in *Drosophila melanogaster*. *Free Radical Research*, 55(8), 919–935. <https://doi.org/10.1080/10715762.2021.1914335>
- O'Connor, E. : C. R. M. B. A. D. P. F. P. (Ed.). (2014a). *Chemistry of Europe's Agricultural Soils, Part A*. Schweizerbart Science Publishers. http://www.schweizerbart.de/publications/detail/isbn/9783510968466/Geologisches_Jahrbuch_Reihe_B_Heft_B102
- O'Connor, E. : C. R. M. B. A. D. P. F. P. (Ed.). (2014b). *Chemistry of Europe's Agricultural Soils, Part B*. Schweizerbart Science Publishers. http://www.schweizerbart.de/publications/detail/isbn/9783510968473/Geologisches_Jahrbuch_Reihe_B_Heft_B103

- Reimann, C., Birke, M., Demetriades, A., Filzmoser, P., O'connor, P., VALERA, P., & Team, G. P. (2014). *Chemistry of Europe's agricultural soils–Part B: General background information and further analysis of the GEMAS data set*.
- Zapletalová, A., Kolenčík, M., Ducsay, L., Vicianová, M., Vician, T., Černý, I., & Bušo, R. (2025). Approach to Selenium Application in Different Soil Concentrations for Encouraged Yield, Distribution, and Biofortification of Common Buckwheat Seeds (*Fagopyrum esculentum* Moench). *Agriculture*, 15(8). <https://doi.org/10.3390/agriculture15080891>
- Zhang, X., Gao, F., Dai, Y., Wang, M., & Liu, J. (2025). Safe-by-design strategies towards bismuth-based nanomaterials in tumor diagnosis and therapy. *Nano Today*, 62, 102714. <https://doi.org/10.1016/j.nantod.2025.102714>
- Zouraris, D., Mavrogiorgis, A., Tsoumanis, A., Saarimäki, L. A., del Giudice, G., Federico, A., Serra, A., Greco, D., Rouse, I., Subbotina, J., Lobaskin, V., Jagiello, K., Ciura, K., Judzinska, B., Mikolajczyk, A., Sosnowska, A., Puzyn, T., Gulumian, M., Wepener, V., ... Afantitis, A. (2025). CompSafeNano project: NanoInformatics approaches for safe-by-design nanomaterials. *Computational and Structural Biotechnology Journal*, 29, 13–28. <https://doi.org/10.1016/j.csbj.2024.12.024>

7. PUBLICATIONS IN THE FIELD OF RESEARCH



UNIVERSITY of
DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN

H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/5/2026.PL
Subject: PhD Publication List

Candidate: Arjun Muthu
Doctoral School: Doctoral School of Food Sciences
MTMT ID: 10094352

List of publications related to the dissertation

Foreign language scientific articles in international journals (6)

1. **Muthu, A.**, Nguyen, H. H. D., Neji, C., Ferroudj, A., Prokisch, J., El-Ramady, H., Béni, Á.:
Determination of hydride-generated selenium in aqueous matrices by modified atomic
fluorescence spectrometry.
J. Food Compos. Anal. 153, 1-10, 2026. ISSN: 0889-1575.
DOI: <http://dx.doi.org/10.1016/j.jfca.2026.109169>
IF: 4.6 (2024)
2. **Muthu, A.**, Nguyen, H. H. D., Ferroudj, A., Prokisch, J., El-Ramady, H., Neji, C., Béni, Á.: Green
Synthesised Carbon Nanodots Using the Maillard Reaction for the Rapid Detection of
Elemental Selenium in Water and Carbonated Beverages.
Nanomaterials. 15 (15), 1-16, 2025. ISSN: 2079-4991.
DOI: <http://dx.doi.org/10.3390/nano15151161>
IF: 4.3 (2024)
3. **Muthu, A.**, Nguyen, H. H. D., Neji, C., El-Ramady, H., Prokisch, J., Béni, Á.: Nanohybrid-based
fluorescent "turn-on" system for selective detection of mercury (II) in aqueous solution.
J. Food Compos. Anal. 148, 1-10, 2025. ISSN: 0889-1575.
DOI: <http://dx.doi.org/10.1016/j.jfca.2025.108653>
IF: 4.6 (2024)
4. **Muthu, A.**, Nguyen, H. H. D., Neji, C., Seresné Törös, G., Ferroudj, A., Atieh, R., Prokisch, J., El-
Ramady, H., Béni, Á.: Nanomaterials for Smart and Sustainable Food Packaging: Nano-
Sensing Mechanisms, and Regulatory Perspectives.
Foods. 14 (15), 2-29, 2025. EISSN: 2304-8158.
DOI: <https://doi.org/10.3390/foods14152657>
IF: 5.1 (2024)
5. Prokisch, J., Sári, D., **Muthu, A.**, Nagy, A., El-Ramady, H., Abdalla, N., Dobránszki, J.:
Biotechnology of Nanofiber in Water, Energy, and Food Sectors.
Agronomy-Basel. 13 (11), 1-31, 2023. EISSN: 2073-4395.
DOI: <http://dx.doi.org/10.3390/agronomy13112734>
IF: 3.3





6. **Muthu, A.**, Sári, D., Ferroudj, A., El-Ramady, H., Béni, Á., Badgar, K., Prokisch, J.: Microbial-Based Biotechnology: Production and Evaluation of Selenium-Tellurium Nanoalloys. *Appl. Sci.-Basel*. 13 (21), 1-14, 2023. ISSN: 2076-3417.
DOI: <http://dx.doi.org/10.3390/app132111733>
IF: 2.5

Foreign language abstracts (4)

7. **Muthu, A.**, Neji, C., Nguyen, H. H. D., Prokisch, J., Béni, Á.: Application of Machine learning for predicting survival outcomes of nano selenium dosage in different in-vivo and in-vitro models. In: Reshaping Global Food Systems / (eds.) Dongxiao Sun-Waterhouse, Paul Finglas, Sian Astley, Nandika Bandara, Elsevier, Glasgow, 21, 2025.
8. **Muthu, A.**, Nguyen, H. H. D., Prokisch, J., Béni, Á.: Biosynthesis of Selenium and Tellurium nanoparticles and their alloys using *Lactobacillus casei*: a sustainable approach to functional nutraceuticals.
In: 1st International Congress on Sustainable Food, Green Chemistry and Human Nutrition : GreenNutrifood2025 : Book of abstracts. Eds.: Filip Dujmić, Sven Karlović, Antonela Ninčević Grassino, University of Zagreb, Zagreb, 38-38, 2025. ISBN: 9789536893225
9. **Muthu, A.**, Nguyen, H. H. D., Prokisch, J., Béni, Á.: Rapid estimation of elemental selenium (Se0) using carbon nanodots.
In: Abstract Book / s.n, Elsevier, Glasgow, 102-102, 2025.
10. **Muthu, A.**, Ferroudj, A., Sári, D., Béni, Á., El-Ramady, H., Prokisch, J.: Production methods for making nano Selenium, nano Tellurium and nano alloys, as potential materials in the food and pharma industry.
In: Táplálkozástudományi Kutatások : XI. PhD konferencia programja és az előadások összefoglalói. Szerk.: Bíró Lajos, Gelencsér Éva, Lugasi Andrea, Rurik Imre, Simonné Sarkadi Livia, Magyar Táplálkozástudományi Társaság, Budapest, 13, 2023. ISBN: 9786155606144

List of other publications

Foreign language international book chapters (1)

11. Nguyen, H. H. D., **Muthu, A.**, Béni, Á., Prokisch, J.: Analytical Techniques for Characterization of Carbon Dots.
In: Carbon Nanodots - Synthesis and Applications in Health, Food and Agriculture / (eds.) Mahendra Rai, Sushama Talegaonkar, József Prokisch, Springer Nature, Philadelphia, 121-147, 2026. ISBN: 9789819547050





Foreign language scientific articles in Hungarian journals (1)

12. Ferroudj, A., **Muthu, A.**, Sári, D., Seresné Törös, G., Béni, Á., El-Ramady, H., Prokisch, J.:

Developing an egg model for selenium nanoparticle testing.

Acta Aliment. 2025, 1-10, 2025. ISSN: 0139-3006.

DOI: <http://dx.doi.org/https://doi.org/10.1556/066.2025.00125>

IF: 1 (2024)

Foreign language scientific articles in international journals (20)

13. Prokisch, J., Nguyen, H. H. D., **Muthu, A.**, Posta, J., Béni, Á.: Quantitative determination of

carbon nanodots in processed foods using HPLC-SEC with fluorescence detection.

Microchem J. 224, 1-9, 2026. ISSN: 0026-265X.

DOI: <http://dx.doi.org/10.1016/j.microc.2026.117773>

IF: 5.1 (2024)

14. Neji, C., **Muthu, A.**, Huzsvai, L., Ungai, D., Seres, E., Prokisch, J., Máthé, E., Sipos, P.:

Assessing data to optimize soybean protein extraction.

LWT-Food Sci. Technol. 217, 1-12, 2025. ISSN: 0023-6438.

DOI: <http://dx.doi.org/10.1016/j.lwt.2025.117401>

IF: 6.6 (2024)

15. Neji, C., **Muthu, A.**, Ungai, D., Seres, E., Domokos-Szabolcsy, É., Prokisch, J., Máthé, E., Sipos, P.:

Assessing Deep Eutectic Solvent Efficiency by Optimizing Soybean Protein Extraction.

Food Bioprocess Technol. 18 (11), 9423-9437, 2025. ISSN: 1935-5130.

DOI: <http://dx.doi.org/10.1007/s11947-025-03973-9>

IF: 5.8 (2024)

16. Nguyen, H. H. D., **Muthu, A.**, Elsakhawy, T., Sheta, M., Abdalla, N., El-Ramady, H., Prokisch, J.:

Carbon Nanodots-Based Sensors: A Promising Tool for Detecting and Monitoring Toxic Compounds.

Nanomaterials. 15 (10), 1-27, 2025. ISSN: 2079-4991.

DOI: <http://dx.doi.org/10.3390/nano15100725>

IF: 4.3 (2024)

17. Ferroudj, A., **Muthu, A.**, Sári, D., Seresné Törös, G., Béni, Á., Czeglédi, L., Knop, R., El-Ramady,

H., Prokisch, J.: Comparative Study of Red and Grey Selenium Nanoparticles on Organ-Specific Selenium Deposition and Growth Performance in Japanese Quails.

Nanomaterials. 15 (11), 1-14, 2025. ISSN: 2079-4991.

DOI: <https://doi.org/10.3390/nano15110801>

IF: 4.3 (2024)





18. Ferroudj, A., **Muthu, A.**, Pesti-Asbóth, G., Sári, D., Seresné Törös, G., Béni, Á., Czeglédi, L., Knop, R., El-Ramady, H., Prokisch, J.: Dietary Red and Grey Selenium Nanoparticles: Effects on Tissue Selenium Distribution, Antioxidant Capacity, and Retention in Japanese Quails. *Antioxidants*. 15 (1), 1-17, 2025. EISSN: 2076-3921.
DOI: <https://doi.org/10.3390/antiox15010004>
IF: 6.6 (2024)
19. Nguyen, H. H. D., El-Ramady, H., Seresné Törös, G., **Muthu, A.**, Elsakhawy, T., Abdalla, N., Alibrahem, W., Kharrat Helu, N., Prokisch, J.: Food-Derived Carbon Dots: Formation, Detection, and Impact on Gut Microbiota. *Foods*. 14 (17), 1-21, 2025. EISSN: 2304-8158.
DOI: <http://dx.doi.org/10.3390/foods14172980>
IF: 5.1 (2024)
20. Seresné Törös, G., El-Ramady, H., Nguyen, H. H. D., Alibrahem, W., Helu, N. K., Atieh, R., **Muthu, A.**, Jevcsák, S., Semsey, D., Abdalla, N., Elsakhawy, T., Tóth, F. A., Nagy, P. T., Prokisch, J.: Green-Synthesized Nanomaterials from Edible and Medicinal Mushrooms: A Sustainable Strategy Against Antimicrobial Resistance. *Pharmaceutics*. 17 (11), 1-26, 2025. EISSN: 1999-4923.
DOI: <https://doi.org/10.3390/pharmaceutics17111388>
IF: 5.5 (2024)
21. Prokisch, J., Seresné Törös, G., Nguyen, H. H. D., **Muthu, A.**, Labidi, S., Sheta, M., El-Ramady, H.: Sustainable Approach of Carbon Nanodots: Agro-Applications for Soil Health. *Egypt. J. Soil Sci.* 65 (1), 639-657, 2025. ISSN: 0302-6701.
DOI: <http://dx.doi.org/10.21608/ejss.2025.353791.1969>
IF: 3.4 (2024)
22. Seresné Törös, G., Gulyás, G., El-Ramady, H., Alibrahem, W., **Muthu, A.**, Gangakhedkar, P., Atieh, R., Prokisch, J.: Sustainable nanotechnology strategies for modulating the human gut microbiota. *Int. J. Mol. Sci.* 26 (12), 1-26, 2025. ISSN: 1661-6596.
DOI: <https://doi.org/10.3390/ijms26125433>
IF: 4.9 (2024)
23. Semsey, D., Nguyen, H. H. D., Seresné Törös, G., **Muthu, A.**, Labidi, S., El-Ramady, H., Béni, Á., Rai, M., Prokisch, J.: Analysis of Fluorescent Carbon Nanodot Formation during Pretzel Production. *Nanomaterials*. 14 (13), 1-11, 2024. ISSN: 2079-4991.
DOI: <http://dx.doi.org/10.3390/nano14131142>
IF: 4.3





24. Prokisch, J., Nguyen, H. H. D., **Muthu, A.**, Ferroudj, A., Singh, A., Agrawal, S., Rajput, V. D., Ghazaryan, K., El-Ramady, H., Rai, M.: Carbon Nanodot-Microbe-Plant Nexus in Agroecosystem and Antimicrobial Applications. *Nanomaterials*. 14 (15), 1-37, 2024. ISSN: 2079-4991. DOI: <http://dx.doi.org/10.3390/nano14151249> IF: 4.3
25. Nguyen, H. H. D., **Muthu, A.**, El-Ramady, H., Béni, Á., Prokisch, J.: Detection and formation of fluorescent carbon nanodots in coffee brews and its relationship with other compositions. *J. Food Compos. Anal.* 132, 1-10, 2024. ISSN: 0889-1575. DOI: <http://dx.doi.org/10.1016/j.jfca.2024.106347> IF: 4.6
26. Prokisch, J., Seresné Törőss, G., Nguyen, H. H. D., Neji, C., Ferroudj, A., Sári, D., **Muthu, A.**, Brevik, E. C., El-Ramady, H.: Nano-Food Farming: Toward Sustainable Applications of Proteins, Mushrooms, Nano-Nutrients, and Nanofibers. *Agron. J.* 14, 1-30, 2024. ISSN: 0002-1962. DOI: <http://dx.doi.org/https://doi.org/10.3390/agronomy14030606> IF: 2
27. Nguyen, H. H. D., **Muthu, A.**, El-Ramady, H., Daróczi, L., Nagy, L., Kéki, S., Béni, Á., Csarnovics, I., Prokisch, J.: Optimization of Extraction Conditions to Synthesize Green Carbon Nanodots from Maillard Reaction. *Mater. Adv.* 5, 3499-3505, 2024. EISSN: 2633-5409. DOI: <http://dx.doi.org/10.1039/D4MA00037D> IF: 4.7
28. Bommasamudram, J., **Muthu, A.**, Devappa, S.: Studies on different polysaccharides used as carrier material for spray-drying of synbiotics and its viability under different storage conditions. *Bioactive Carbohydrates and Dietary Fibre*. 31, 1-11, 2024. ISSN: 2212-6198. DOI: <http://dx.doi.org/10.1016/j.bcdf.2024.100421> IF: 3.6
29. Bommasamudram, J., **Muthu, A.**, Devappa, S.: Effect of prebiotics on thermally acclimatized lactobacilli cultures and their application as synbiotics in RTD fruit drinks. *3 Biotech*. 13 (9), 1-10, 2023. ISSN: 2190-572X. DOI: <http://dx.doi.org/10.1007/s13205-023-03737-2> IF: 2.6
30. Sári, D., Ferroudj, A., **Muthu, A.**, Béni, Á., Jamalifard, R., Prokisch, J., El-Ramady, H., Elsakhawy, T., Omara, A. E. D., Brevik, E. C.: Nano-Enabled Agriculture Using Nano-Selenium for Crop Productivity: What Should be Addressed More? *EBSS*. 7, 85-99, 2023. EISSN: 2536-9423. DOI: <http://dx.doi.org/10.21608/jenvbs.2023.205664.1215>





31. El-Ramady, H., Abdalla, N., Sári, D., Ferroudj, A., **Muthu, A.**, Prokisch, J., Fawzy, Z., Brevik, E. C., Solberg, S. Ø.: Nanofarming: Promising Solutions for the Future of the Global Agricultural Industry.
Agronomy-Basel. 13 (6), 1-32, 2023. EISSN: 2073-4395.
DOI: <http://dx.doi.org/10.3390/agronomy13061600>
IF: 3.3
32. Bommasamudram, J., **Muthu, A.**, Devappa, S.: Effect of sub-lethal heat stress on viability of *Lactocaseibacillus casei* N in spray-dried powders.
LWT-Food Sci. Technol. 155, 1-9, 2022. ISSN: 0023-6438.
DOI: <http://dx.doi.org/10.1016/j.lwt.2021.112904>
IF: 6

Foreign language abstracts (3)

33. Ferroudj, A., **Muthu, A.**, Sári, D., Seresné Törös, G., Béni, Á., Czeglédi, L., Knop, R., El-Ramady, H., Prokisch, J.: Impact of Red and Grey Nano-Selenium Supplementation on Growth and Feed Efficiency in Japanese quails.
In: *Fostering the Transition to Sustainable Food Systems: Embracing Novelty and Overcoming Challenges : abstracts book*
34. Ferroudj, A., **Muthu, A.**, Sári, D., Béni, Á., Czeglédi, L., Knop, R., Prokisch, J.: Spectroscopic characterization of nano-selenium and its dietary effects on quail growth and organ selenium uptake.
In: *Book of abstracts of the 30th International Conference Krmiva 2025/ Zvonko Antunovic, Zlatko Janjecic, Krmiva d.o.o. Zagreb, Zagreb, , 2025, (ISSN 1847-2370)*
35. Nguyen, H. H. D., **Muthu, A.**, El-Ramady, H., Prokisch, J.: Detection of Carbon nanodots in black foods.
In: *Táplálkozástudományi Kutatások : XI. PhD konferencia programja és az előadások összefoglalói. Szerk.: Bíró Lajos, Gelencsér Éva, Lugasi Andrea, Rurik Imre, Simonné Sarkadi Livia, Magyar Táplálkozástudományi Társaság, Budapest, 16, 2023. ISBN: 9786155606144*

Total IF of journals (all publications): 112,4

Total IF of journals (publications related to the dissertation): 24,4

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.



23 April, 2026