FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 9, May, 2026, pp 126 – 131
DOI: https://doi.org/10.33003/fjs-2026-1009-5340

Development and Field Demonstration of a qPCR Panel for Heavy-Metal Detoxification Genes in Tomato from Irrigated Sites in Kano, Nigeria

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ABSTRACT

Heavy-metal contamination of wastewater-irrigated farmland in the Kano River Basin notably lead, cadmium, chromium, nickel, copper and zinc has been characterised repeatedly at the soil and tissue level, but local molecular tools for monitoring how crops respond to it are lacking. This paper presents the development, validation and first field application of a multi-gene quantitative real-time PCR (qPCR) panel targeting phytochelatin synthase (PCS1) and glutathione reductase (GR-1) detoxification related genes with actin (ACT) as the reference gene in tomato (*Solanum lycopersicum*). Primer performance was established by serial dilution standard curves and melt-curve specificity analysis: ACT amplified with an efficiency of 90.6% (slope -3.57, R^2 = 0.998), PCS1 produced a single specific product (melt temperature 83.5 ± 0.0 °C; R^2 = 0.971), and GR-1 produced a single product at 81.1 ± 0.2 °C. Metallothionein-2 (MT-2) primers were excluded on specificity grounds. The validated panel was then applied to root and shoot tissue of field-grown tomato sampled in December 2025 from three contaminated sites along a rural-to-industrial gradient Challawa, Wudil and Dambatta and a control at Bayero University, Kano. Exposure was from natural field contamination rather than controlled dosing. GR-1 was preferentially expressed in roots and most strongly at the most contaminated site while PCS1 expression was more evident in shoot tissue patterns consistent with the metal partitioning documented in the same agroecosystem in earlier studies indicating that the panel returns biologically coherent signal. The panel provides a locally validated, low-cost tool for transcript-level monitoring of metal detoxification in field crops.

Received: 10 June 2026

Accepted: 19 June 2026

Published: !9 June 2026

Keywords: Assay validation, Heavy-metal monitoring, qPCR panel, *Solanum lycopersicum*

INTRODUCTION

Wastewater irrigation in the farmland surrounding the Kano River Basin has long been associated with the accumulation of heavy metals such as lead, cadmium, chromium, nickel, copper and zinc in soils and crops, the metals being carried in largely untreated effluents from tanneries, textile mills and metal workshops (Akan *et al*., 2009; Hamidu *et al*., 2021). The resulting contamination of locally grown produce, and its public-health implications, have been documented in a series of studies across the region (Lawal and Audu, 2011; Edogbo *et al*., 2020; Isiuku and Enyoh, 2020; Muktar *et al*., 2021). In an earlier phase of the present work, we quantified soil contamination indices and metal partitioning in an edible plant(tomato) and in the non-edible Dodonaea viscosa across an industrial gradient in Kano, confirming a steep contamination gradient and tissue-level metal uptake in the edible crop (Yusuf *et al*., 2025).

Researchers have done a lot of work measuring heavy metals in the soil and crops of the Kano River Basin. They know how much metal is there and where it builds up but what they do not have is a way to see how the plants themselves are responding to that stress at the molecular level. Plants tolerate heavy metals through defenses like binding them up, storing them away, and fighting the damage metals cause (Hall, 2002; Nagajyoti et al., 2010; Sharma and Dietz, 2009; Hossain and Komatsu, 2012; Emamverdian *et al*., 2015). The genes that control these responses are measurable. Two genes are particularly useful. Phytochelatin synthase (PCS) makes small metal-binding peptides that grab onto cadmium, lead, and copper (Cobbett, 2000; Mejare and Bulow, 2001; Kisa, 2019), while glutathione reductase (GR) helps recycle a key antioxidant that protects the plant from metal damage and also supplies the raw material for making those peptides (Gill and Tuteja, 2010; Hasanuzzaman et al., 2019). In tomato plants, both PCS1 and GR-1 have been shown to respond when metals are present (Kisa, 2019; Bolukbasi, 2021), as have other stress-related genes under abiotic stress (Tombuloglu *et al*., 2012), making them logical targets for a simple, practical monitoring assay.

Translating such targets into a usable monitoring tool, requires more than just a primer sequence. A dependable qPCR must be validated under local laboratory conditions. Its amplification efficiency, standard-curve linearity and product specificity must be established before it can be applied with confidence (Bustin et al., 2009; Livak and Schmittgen, 2001). For the contaminated agroecosystems of northern Nigeria, no such locally validated panel for detoxification-gene monitoring has been reported in literature, which leaves the molecular dimension of a well characterized contamination problem effectively unmonitorable in practice.

Tomato (*Solanum lycopersicum*) was chosen as the test crop for practical reasons. It is one of the most widely cultivated irrigated vegetables in the Kano River Basin, it has a short growing cycle that allows responses to be captured within a single season, and it is known to take up and accumulate heavy metals in its tissues, making it both an economically important crop and a sensitive indicator of contamination. Quantitative real-time PCR was chosen as the platform because it is quantitative, relatively inexpensive once established, and, in a multi-gene panel format, allows several detoxification genes to be measured together from the same sample an efficiency that suits a low-resource monitoring setting.

This study addresses that practical gap. Its objective is to develop and validate a multi-gene qPCR panel (PCS1, GR-1, ACT) for tomato and to demonstrate its application on field tissue collected across the Kano contamination gradient established in our earlier work. The emphasis is on the tool validation and its capacity to return biologically meaningful signal from real field samples rather than on hypothesis-testing of expression differences, which is the intended next stage. In providing this validated panel, the study delivers the molecular monitoring capability that the regional literature has so far lacked, and fulfils the molecular-mechanism objective of the wider project.

MATERIALS AND METHODS

Panel Design and Gene Targets

The panel comprised of two detoxification-related target genes and one reference gene. The targets selected were phytochelatin synthase (PCS1), the enzyme of phytochelatin biosynthesis, and glutathione reductase (GR-1) which is a key enzyme of the ascorbate glutathione antioxidant cycle. Actin (ACT) was used as the reference gene for normalisation. Primer sequences were adopted from previously published sources. The PCS1 primers were those reported by Kisa (2019) and the ACT reference-gene primers those used by Bolukbasi (2021) and Kisa (2019): PCS1 forward 5′-TGCTAGCATTTGTTGCCAAG-3′, reverse 5′-ACGTAGGGACCAGAACATCG-3′; ACT forward 5′-GGGATGGAGAAGTTTGGTGGTGG-3′, reverse 5′-CTTCGACCAAGGGATGGTGTAGC-3′. The GR-1 primers were those reported by Bolukbasi (2021). Primer suitability was checked with reference to standard primer-design criteria (Rozen & Skaletsky, 2000). Metallothionein-2 (MT-2) primers were additionally screened as a candidate panel member.

Field Samples for Demonstration

Tomato (*Solanum lycopersicum*) tissue was collected from three wastewater-irrigated sites along the contamination gradient characterised in our previous study (Yusuf *et al.*, 2025). The samples were collected from Challawa, Wudil and Dambatta together with a control site at Bayero University, Kano (BUK). At these sites the irrigation soils carry an industrial heavy-metal burden lead, cadmium, chromium, nickel, copper and zinc documented in our earlier survey (Yusuf et al., 2025), in which mean soil lead reached 106.6 mg/kg and cadmium 16.5 mg/kg at the most contaminated site (Challawa), declining to 15.6 mg/kg and 0.4 mg/kg at the control, with the pollution load index falling from 8.15 at Challawa to 0.96 at the control. The tomato analysed here was therefore exposed to this multi-metal field contamination rather than to a single dosed metal. In each site, plants were separated into root and shoot tissue, giving eight tissue site samples for assay demonstration. Tomato tissue was collected from the field sites in December 2025. The samples were therefore held in desiccated storage for approximately four months before laboratory processing in March 2026, an interval that may affect RNA integrity and is considered in the Limitations. The root and shoot tissue were sampled and preserved by desiccation and stored in labelled sample bags prior to transportation. One composite sample for root and shoot were prepared per site giving eight samples (four sites × two tissues) for analysis. RNA extraction for 8 samples and cDNA synthesis 8 reactions were performed commercially at Inqaba Biotec West Africa Ltd, Ibadan, Nigeria using their standard kit-based extraction and reverse-transcription protocols.

qPCR Conditions

Reactions were run on a CFX96 Real-Time PCR System (Bio-Rad) with SYBR-based detection using Luna Universal qPCR Master Mix. Reactions were prepared using the provider's standard Luna Universal reaction setup and run under a two-step protocol incorporating a 60 °C combined annealing/extension step which was run and completed 16 March 2026 using CFX Manager v3.1. Two technical replicates were run per sample and a no-template control (NTC) was included for each gene.

Validation Criteria and Data Treatment

Assay performance was assessed by three validation criteria : amplification efficiency, standard-curve linearity and product specificity. Amplification efficiency was calculated from the slope of a serial-dilution standard curve as $E = 10^{\left(\frac{-1}{\text{slope}}\right)} - 1$ and expressed as a percentage (% efficiency = E × 100). Standard-curve linearity was assessed by the coefficient of determination (R^2) while product specificity was assessed by melt-curve analysis where a single, sharp dissociation peak at a consistent melt temperature indicates a specific product. For the field demonstration, mean quantification-cycle (Cq) values were calculated from the two technical replicates and relative expression was estimated by the comparative $2^{-\Delta\Delta \text{Ct}}$ method (Livak and Schmittgen, 2001), normalised to ACT and expressed relative to the corresponding control tissue. As the demonstration used a single field batch with technical replication due to cost constraints, relative-expression values are presented as descriptive indicators of assay response, not as statistically tested expression differences.

RESULTS AND DISCUSSION

Standard-curve Validation of the Reference and Target Assays

The reference assay ACT amplified with high efficiency and excellent linearity across the dilution series with a slope -3.57, $R^2 = 0.998$ and efficiency 90.6%. This falls within the optimal 90–110% range and confirming its suitability as the normaliser as shown Figure 1. The PCS1 target assay showed strong linearity $R^2 = 0.971$. Its calculated efficiency of 117.7% as shown in figure exceeded the optimal window and is addressed in the Limitations. Validation parameters are summarised in Table 1.

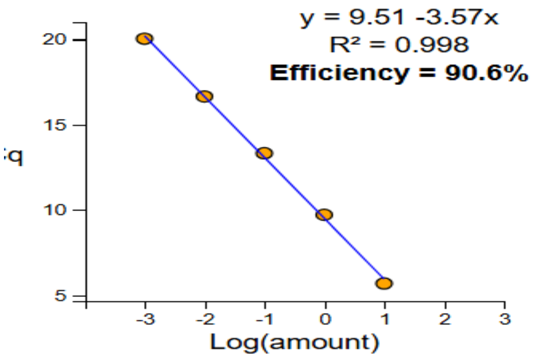

Figure 1: ACT Standard Curve (Slope -3.57, R² = 0.998, Efficiency 90.6%)

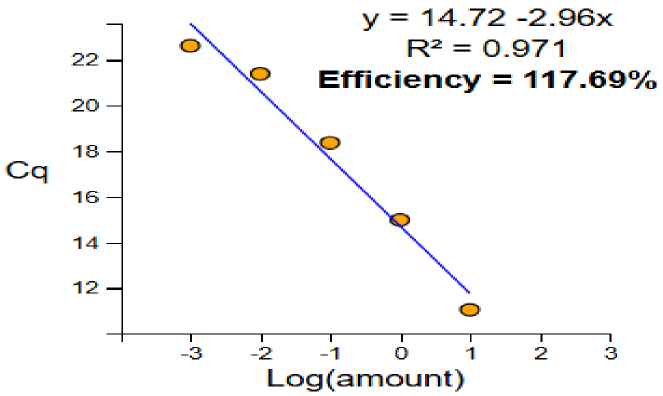

Figure 2: PCS1 Standard Curve (Slope -2.96, R² = 0.971, Efficiency 117.7%)

Table 1: qPCR Panel Validation Parameters. Melt Temperatures are Mean ± SD across Field Samples. n/d = not Determined

Gene	Role	Slope	R²	Eff. (%)	Melt T (°C)
ACT	Reference	-3.57	0.998	90.6	81.1 ± 0.9
PCS1	Target (chelation)	-2.96	0.971	117.7	83.5 ± 0.0
GR-1	Target (antioxidant)	n/d	n/d	n/d	81.1 ± 0.2
MT-2	Screened – excluded	n/d	n/d	n/d	non-specific

Product Specificity Across all Field Samples

Across all eight field samples, each target produced a single melt peak at a characteristic, highly reproducible temperature: PCS1 at 83.5 ± 0.0 °C and GR-1 at 81.1 ± 0.2 °C, with ACT at 81.1 ± 0.9 °C (Figure 3). The near-zero variance of the PCS1 and GR-1 melt temperatures demonstrates consistent, specific amplification of a single product in every sample, confirming that the panel performs reliably on field-derived material and not only on clean standards.

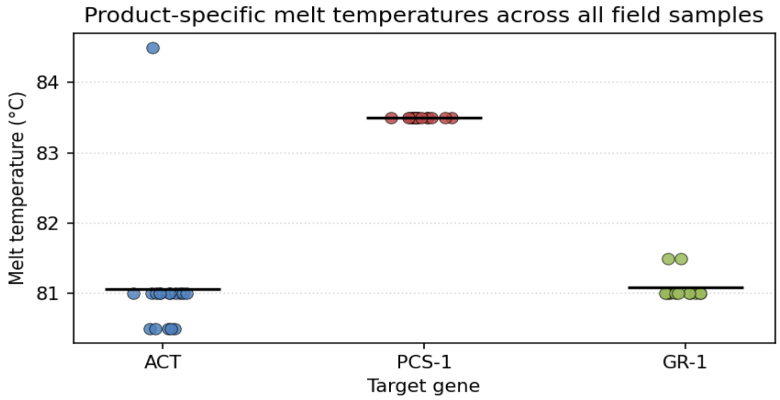

Figure 3. Product-specific Melt Temperatures of each Target Across all Field Samples; Horizontal bars show Means. Tight Clustering Indicates Single-product Specificity

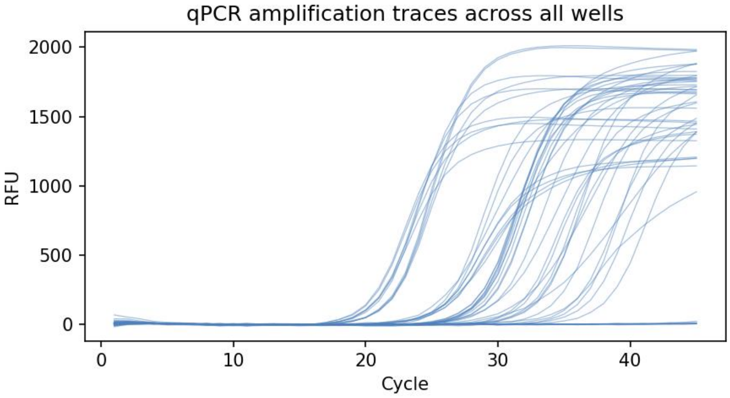

Figure 4: qPCR Amplification Traces Across all wells, Illustrating Consistent Amplification Behaviour of the Panel

MT-2 Screening and Exclusion

Under identical conditions, MT-2 primers produced an additional dissociation peak alongside the expected product and lacked the clean amplification behaviour of the retained assays, indicating non-specific amplification. MT-2 was therefore excluded from the validated panel and is flagged for primer redesign a documented design decision rather than a discarded result.

Field Demonstration: Panel Response Across the Contamination Gradient

The panel as shown in Table 2 returned interpretable Cq values for the reference and both targets on the eight field samples. The reference gene ACT amplified at comparable levels across samples, while the targets varied by site and tissue in a biologically coherent direction. Relative-expression estimates as shown in Figures 5 and 6 showed GR-1 preferentially expressed in roots and most strongly at the most contaminated site Challawa. It showed declining toward the less contaminated sites while PCS1 expression was more evident in shoot tissue across the contaminated sites. These directions match the metal-partitioning patterns reported for the same agroecosystem (Yusuf *et al.*, 2025) and demonstrate that the panel detects expected detoxification biology in field material.

Table 2: Mean Cq Values from the Field Demonstration (Mean of two Technical Replicates). n.d. = not Detected

Sample (site – tissue)	ACT Cq	PCS1 Cq	GR-1 Cq
Control – Root (BUK)	21.23	21.71	32.97
Control – Shoot (BUK)	29.72	39.16	33.83
Challawa – Root	27.28	34.06	31.19
Challawa – Shoot	22.61	29.40	27.04
Wudil – Root	27.35	27.71	37.05
Wudil – Shoot	21.81	22.71	29.43
Dambatta – Root	29.49	30.04	37.19
Dambatta – Shoot	34.69	37.36	n.d.

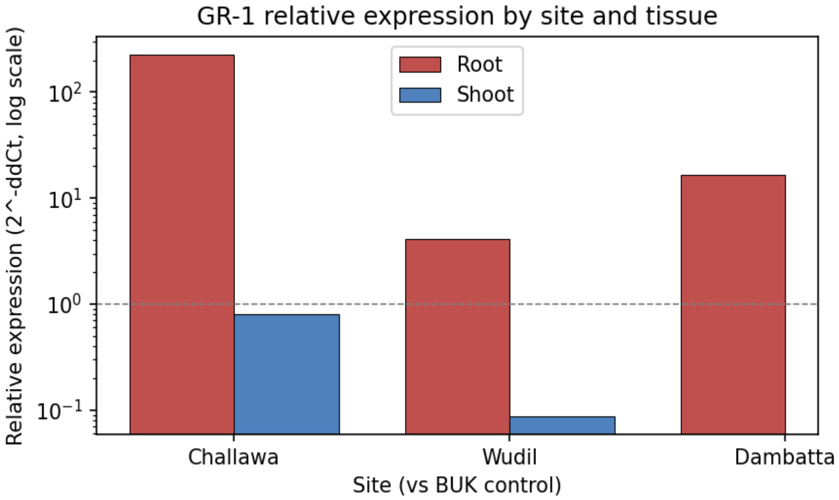

Figure 5: GR-1 Relative Expression (2^-ΔΔCt, log Scale) across the Field Gradient, Demonstrating Panel Response

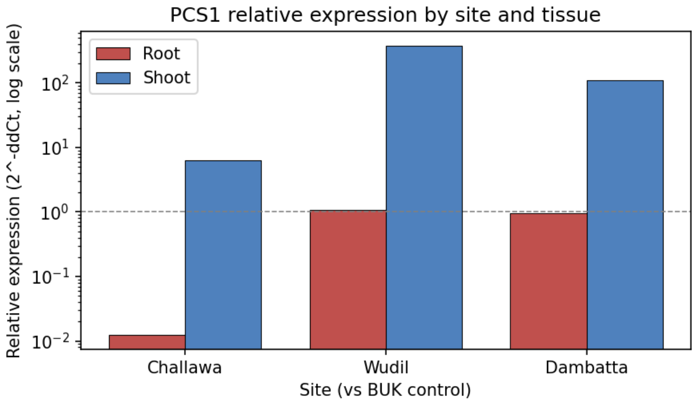

Figure 6. PCS1 Relative Expression (2^-ΔΔCt, Log Scale) across the Field Gradient. Large Shoot Estimates Indicate Direction, not Exact Magnitude

Discussion

The principal outcome of this study is a validated, locally established qPCR panel for monitoring heavy-metal detoxification genes in tomato. The reference assay (ACT) met all standard validation criteria and both targets produced single, highly reproducible melt products across every field sample tested with near-zero variance in the PCS1 and GR-1 melt temperatures. This is a clear demonstration of specificity on real, field-derived RNA rather than on idealized standards alone. After all, a monitoring tool is only as useful as its behaviour on messy field material, and on that test this panel performed well.

The panel recovered biologically sensible signal and the direction of that signal can be interpreted against both the soil-contamination data and earlier molecular work. The two genes responded in a way that tracked the field contamination. That is, the strongest GR-1 response occurred at Challawa, the site with the highest soil lead, cadmium and chromium and the highest pollution load index (8.15), and the response weakened toward the less contaminated sites, indicating that the transcriptional signal scales with the metal burden the plants experienced. GR-1 was expressed most strongly in roots at the most contaminated site. This fits the antioxidant role of the ascorbate–glutathione cycle at the principal site of metal uptake. Gill and Tuteja (2010) and Hasanuzzaman et al. (2019) describe glutathione reductase as central to managing metal-induced oxidative stress, and Bolukbasi (2021) reported comparable cadmium-responsive modulation of GR-1 in tomato, so the root-dominated GR-1 response observed here is consistent with the antioxidant function those studies describe. The shoot-biased PCS1 signal points the other way toward chelation in aerial tissue. Cobbett (2000) established that phytochelatin synthase drives metal chelation, and Kisa (2019) found PCS1 induced under metal stress in tomato consistent with the shoot pattern seen in this work. In a nutshell, GR-1 was expressed mainly in the roots and PCS1 mainly in the shoots. This tissue-specific division mirrors the organ-specific detoxification responses reported by other workers and shows that the panel can resolve where a response occurs within the plant rather than returning only a single whole-plant signal.

These molecular signals line up with the elemental evidence from the same farmland. Lawal and Audu (2011) and Edogbo et al. (2020) both recorded heavy metals accumulating in vegetables grown under wastewater irrigation around Challawa, and our own earlier survey (Yusuf et al., 2025) found the same upward partitioning of metals into tomato tissue at these sites. The present transcript data add a mechanistic reading to those observations: if a plant is switching on its chelation machinery in the shoots, metals are evidently reaching the shoots, exactly as the partitioning data of Yusuf et al. (2025) indicated. The panel therefore provides a molecular readout that mirrors, and could in time complement, conventional elemental testing for food safety. The exclusion of MT-2 on specificity grounds which is documented here rather than buried, illustrates the kind of design discipline that makes such a panel reliable and trustworthy.

The contribution is a low-cost, locally validated panel that returns interpretable detoxification-gene signal from field tomato, ready to be deployed in the replicated, dose-controlled studies that the region's contamination problem warrants. The demonstration reported here defines the panel's behaviour and the response patterns worth pursuing. This makes it the foundation for that quantitative work rather than a substitute for it.

A few limitations bound these results. The field application rested on a single biological batch per site tissue with technical replication, so the relative-expression values indicate the direction of the panel's response rather than statistically tested differences, and replicated biological sampling is the clear next step. The PCS1 amplification efficiency of 117.7% exceeded the optimal range despite confirmed linearity, so quantitative PCS1 comparisons should follow standard-curve re-optimisation; a small number of wells did not amplify, and the roughly four-month desiccated-storage interval before processing may have affected RNA recovery. Normalisation also relied on a single reference gene, and the multi-metal field exposure means the panel reports an integrated response rather than the effect of any one metal.

CONCLUSION

This study developed, validated and field-demonstrated a multi-gene qPCR panel for monitoring heavy-metal detoxification in tomato from the wastewater-irrigated farmland of the Kano River Basin. The panel met reference-gene validation criteria, amplified both targets as single specific products across all field samples, and returned biologically coherent signal root-dominant GR-1 expression strongest at the most contaminated site, and shoot-biased PCS1 expression consistent with established detoxification biology and with prior elemental evidence from the same sites. The panel provides the locally validated molecular monitoring capability that the region has lacked, fulfils the objective of investigating molecular detoxification mechanisms, and establishes a clear foundation for replicated, dose-controlled studies.

Building on this foundation, future work should deploy the panel with full biological replication to permit statistical testing of expression differences, re-optimise the PCS1 standard curve and add a second reference gene for quantitative deployment, re-evaluate MT-2 with redesigned primers, and apply the panel in controlled, dose-graded experiments to resolve metal-specific responses.

REFERENCES

Akan, J. C., Abdulrahman, F. I., Ogugbuaja, V. O., and Ayodele, J. T. (2009). Heavy metals and anion levels in some samples of vegetables grown within the vicinity of Challawa industrial area, Kano State, Nigeria. *American Journal of Applied Sciences, 6*(3), 534–542.

Bolukbasi, E. (2021). Expression analysis of some stress-related genes induced by cadmium on tomato (Solanum lycopersicum L.) plants. *Hittite Journal of Science and Engineering, 8*(4), 339–345.

Bustin, S. A., Benes, V., Garson, J. A., *et al.* (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry, 55*(4), 611–622.

Cobbett, C. S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology, 123*(3), 825–832.

Edogbo, B., Okolocha, E., Maikai, B., Aluwong, T., and Uchendu, C. (2020). Risk analysis of heavy metal contamination in soil, vegetables and fish around Challawa area in Kano State, Nigeria. *Scientific African, 7*, e00281.

Emamverdian, A., Ding, Y., Mokhberdoran, F., and Xie, Y. (2015). Heavy metal stress and some mechanisms of plant defense response. *The Scientific World Journal, 2015*, 756120.

Gill, S. S., and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909–930.

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany, 53*(366), 1–11.

Hamidu, H., Abubakar, S., Bala, A., *et al.* (2021). Heavy metals pollution indexing, geospatial and statistical analysis of groundwater around Challawa and Sharada industrial areas, Kano, Nigeria. *SN Applied Sciences, 3*, 1–18.

Hasanuzzaman, M., Bhuyan, M. H. M., Anee, T. I., Parvin, K., and Nahar, K. (2019). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants, 8*(9), 384.

Hossain, Z., and Komatsu, S. (2012). Contribution of proteomic studies towards understanding plant heavy metal stress response. *Frontiers in Plant Science, 3*, 310.

Isiuku, B. O., and Enyoh, C. E. (2020). Monitoring and modeling of heavy metal contents in vegetables collected from markets in Imo State, Nigeria. *Environmental Monitoring and Assessment, 192*(7), 446.

Kisa, D. (2019). Responses of phytochelatin and proline-related genes expression associated with heavy metal stress in Solanum lycopersicum. *Acta Botanica Croatica, 78*(1), 9–16.

Lawal, A. O., and Audu, A. A. (2011). Analysis of heavy metals found in vegetables from some irrigated gardens in Kano State, Nigeria. *Journal of Environmental Chemistry and Ecotoxicology, 3*(6), 142–148.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. *Methods, 25*(4), 402–408.

Mejare, M., and Bulow, L. (2001). Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends in Biotechnology*, 19(2), 67–73.

Muktar, A. A., Alhassan, A. J., Atiku, M. K., Pedro, S. L., and Wudil, A. M. (2021). Assessment of toxicological indices of some heavy metals in soil, cabbage and lettuce samples grown along some rivers in Kano State, Nigeria. *Dutse Journal of Pure and Applied Sciences*, 7(4a), 65–76.

Nagajyoti, P. C., Lee, K. D., and Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters, 8*(3), 199–216.

Rozen, S., & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology, 132*, 365–386.

Sharma, S. S., and Dietz, K. J. (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science, 14*(1), 43–50.

Tombuloglu, H., Semizoglu, N., Sakcali, S., and Kekec, G. (2012). Boron induced expression of some stress-related genes in tomato. *Chemosphere, 86*(4), 433–438.

Yusuf, A., Musa, D. D., and Kutawa, A. B. (2025). Detection and phytoextraction of heavy metals by selected edible and non-edible plants from irrigated sites in Kano Metropolis, Nigeria. *Sahel Journal of Life Sciences FUDMA, 3*(3), 324–332.