Metabolic modifications induced by fertilization with sewage sludge from the Sedrata wastewater treatment plant (North-East Algeria) in potato (*Solanum tuberosum* L.)

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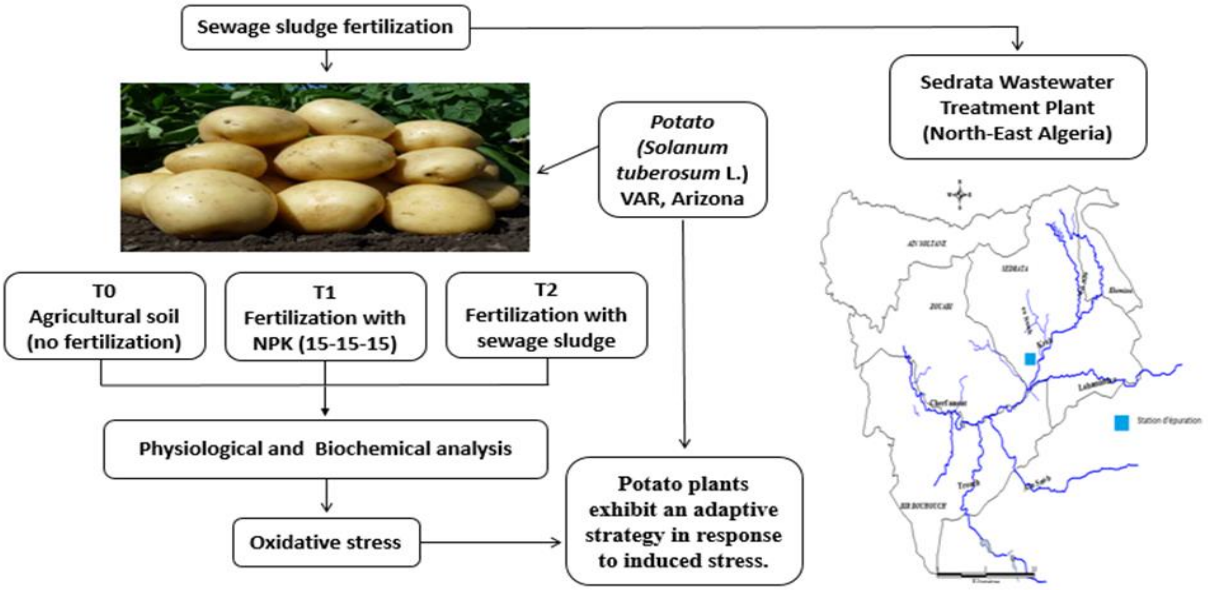
Graphical abstract



Abstract

The use of sewage sludge (SS) in agriculture is a global practice and a unique method of waste management. SS is high in organic matter and fertilizing elements, making it a viable alternative to chemical fertilizers and of significant economic value. To investigate the impact of SS in potato (*Solanum tuberosum* L.) cropping systems, a pot experiment was conducted with three treatments: one with SS and another with the chemical fertilizer NPK (15-15-15) in comparison to the control, to assess the physiological and biochemical responses of the potato by characterizing its defense strategy in the face of the abiotic constraint present in the environment. Unlike carotenoids, Sewage sludge (SS) reduced chlorophyll and protein content. Furthermore, elevated levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were detected, indicating oxidative stress. Increases in reduced glutathione (GSH) and the activities of the antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione-S-transferase (GST) suggest that defense mechanisms are activated in response to this stress. The findings indicate that SS fertilization may be a viable option for potato plants because they exhibit an adaptive strategy in response to induced stress, as evidenced by increased carotenoids and GSH content, as well as various antioxidant levels.

Keywords: Sewage sludge; *Solanum tuberosum*; oxidative stress; antioxidants; adaptative strategy; agriculture; chlorophyll; antioxidant enzymes.

1. Introduction

Sewage sludge (SS), a byproduct of wastewater treatment plants (WWTP), is becoming a global issue as a result of population growth and urbanization. As a result, the use of SS in agriculture is a widespread practice and a highly effective method of sludge disposal. Sewage sludge (SS) amendment could enrich agricultural soils with nutrients, particularly nitrogen and phosphorus (Cerqueira *et al*. 2011), (Cerqueira *et al*. 2012), (Arenas-Lago *et al*. 2013).

The use of SS as fertilizer has grown in popularity due to its high fertilizer value and low cost (Jarausch-Wehrheim *et al*. 2000). SS can be a source of essential plant nutrients such as N, P, K, Mg, Zn, Co, Mn, Fe and B (Candelaria *et al*. 1995), as well as a valuable source of organic matter for cultivated lands in general (Eid et *al*. 2017). However, SS may contain significant amounts of pollutants, including organic and/or heavy metals (HMs) (Rastetter and Gerhardt, 2017). Overall, heavy metal exposure affects many physiological and biochemical activities in plants by increasing the production of reactive oxygen species (ROS), which interact with photosynthetic pigments, proteins, lipids, and nucleic acids, causing membrane damage, lipid peroxidation (Verma and Dubey, 2003), (Nouairi *et al*. 2009), and changes in antioxidative enzyme levels (Ahmed *et al*. 2009).

Plants have used non-enzymatic (GSH, ascorbate, and carotenoids) and enzymatic (SOD, CAT, and APX) antioxidant mechanisms to mitigate and repair ROS-induced damage (Srivastava *et al*. 2009), (Møller, 2001).

Recent studies have highlighted the fundamental role of antioxidant mechanisms in plant tolerance to oxidative (metallic) stress. In *Raphanus sativus* cultivated on substrates amended with various mixtures of sewage sludge and fly ash (SLASH), growth, biomass production, and metabolism were enhanced despite the presence of metals, owing to a pronounced antioxidant response (Sharma and Singh, 2019). Similarly, *Cucumis sativus* exhibited good tolerance to low doses of barium. Still, they showed reduced biomass at higher concentrations, accompanied by oxidative stress mitigated through the activation of CAT, GPX, and APX (Sleimi *et al*. 2021). Moreover, in *Helianthus annuus*, exposure to lanthanum and cerium did not result in visible toxicity, due to strong antioxidant activity and

improved photosynthetic functions (Dridi *et al*. 2022). Finally, *Abelmoschus esculentus* demonstrated greater tolerance to aluminum than to barium, attributable to an effective antioxidant response (SOD, CAT, GR) and detoxification mechanisms involving glutathione and phytochelatins (Kouki *et al*. 2024).

Proper SS application management is critical to maximizing its beneficial effects while minimizing its negative effects (Skjelhaugen, 1999), (Eid *et al*. 2017). Such management should take into account a variety of factors, including the presence of heavy metals and other contaminants, the crop type and its nutrient requirements, the amount of nutrients present in the SS, and the chemical and physical properties of the soil. All of this will help to determine the appropriate rate, time, and method of application (Candelaria *et al*. 1995). Using SS as a fertilizer has been investigated in a variety of plant species. According to (Singh and Agrawal, 2010), adding SS to rice soil may be a good option. Although the benefits of SS are well documented, few studies have looked specifically at how it affects the physiological and biochemical responses of potatoes. This study aims to close the gap by providing specific data on these topics.

2. Material and Methods

2.1. Experimental design

A pot experiment was carried out in the greenhouse of the Department of Biology at the University Mohamed Cherif Messaadia, Souk Ahras. A pot-scale study was chosen because it provided an easy way to evaluate plant production under controlled conditions. This experiment used potato (*Solanum tuberosum* L. var. Arizona) as a plant material. Dehydrated SS was used as a fertilizer, provided from the Sedrata wastewater treatment plant WWTP (Souk-Ahras city, North-Eastern Algeria). It is mainly derived from domestic effluent, with a contribution from craft and agricultural activities.

The purification process adopted is based on the extended aeration activated sludge system, including aerobic biological treatment and secondary decantation, The sludge produced undergoes thickening, followed by natural dehydration on drying beds. The amount of SS used in this experiment was estimated using the potato's nitrogen (N), phosphorus (P), and potassium (K) nutrient requirements

according to ITCMI (Technical Institute for Vegetable and Industrial Crops). Another fertilization with NPK chemical fertilizer (15-15-15) was applied in comparison to the control (unfertilized soil). The experiment involved installing nine identical square pots filled with agricultural soil. This substrate received potato plants (tubers) that were sown at a depth of 15 cm. The experimental design was fully randomized, with three treatments and three replicates per treatment. The treatments were designed according to the following plan:

T0: Agricultural soil without any fertilization (control)

T1: Agricultural soil fertilized with NPK

T2: Agricultural soil fertilized by SS

2.2. Sewage sludge and soil physicochemical analysis

Soil and SS samples were collected, air dried, ground, and sieved (2 mm) for further analysis of pH, organic matter (OM), electrical conductivity (EC), salinity, macronutrients (total N, P, and K), and soluble cations (Mg^{2+} and Ca^{2+}), The samples were also analyzed with wastewater from the WWTP. at the Laboratory of Science and Techniques for Living at the Institute of Agronomic and Veterinary Sciences, University of Souk Ahras. The pH was measured in a 1:5 (sample/water) suspension using a pH meter calibrated with pH 4, 7, and 9.2 reference buffers. Salinity and electrical conductivity were determined using international standards (ISO). For organic matter, the method used was that of (Walkley and Black, 1934). The total nitrogen content was determined using the method described in (Kirk, 1950). A flame photometer was used to measure potassium and phosphorus, as well as the concentrations of soluble cations (Mg^{2+} and Ca^{2+}). Heavy metal concentrations in SS, soil, wastewater and potato (roots and tubers) were assessed using an atomic absorption spectrophotometer (AAS). These heavy metals included lead (Pb), copper (Cu), zinc (Zn), iron (Fe), arsenic (As), manganese (Mn), nickel (Ni), sulfur (S), cobalt (Co), cadmium (Cd), and chromium (Cr).

2.3 Estimations of physiological and biochemical parameters

2.3.1 Chlorophyll analysis

Photosynthetic pigments from fresh potato leaves were extracted in 80% acetone and calcium

bicarbonate ($CaCO_3$) using the method described by (Holden, 1975). The solution was filtered and used to estimate chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids, as previously reported. The pigment contents were calculated using the equations of (Lichtenthaler and Wellburn, 1983).

2.3.2 Proteins assay

Total protein content in potato tubers was determined calorimetrically, as previously reported (Bradford, 1976), with bovine serum albumin as the standard. To perform the assay, add 4 ml of Bradford reagent to 100 µl of protein extract. The mixture was homogenized and allowed to stand for 5 minutes. The absorbance was measured at 595 nm using spectrophotometry against a blank that contained distilled water rather than the extract.

2.3.3 Lipid peroxidation

The evolution of malondialdehyde (MDA) content was used to estimate lipid peroxidation, as described in (Alia *et al*. 1995). Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were used as detection reagents. The absorbance of the TBA-MDA complex was measured at 532 nm against an extraction buffer blank. The optical density was corrected to 600 nm ($\varepsilon = 155\ M^{-1}\ cm^{-1}$).

2.3.4 H_2O_2 concentration

The concentration of hydrogen peroxide (H_2O_2) was determined by homogenizing 500 mg of plant tissue in 5 ml of 0.1% TCA at a rate of 10 ml/g of fresh material. The homogenate was centrifuged at 12000 g for 15 minutes at 4 °C. The supernatant was mixed with 0.5 ml of phosphate buffer (10 mM, pH 7) and 1 ml of potassium iodide (1 M). The absorbance of this mixture was determined at 390 nm (Alexieva *et al*. 2001).

2.4 Evaluation of the antioxidant system

2.4.1 Non-antioxidant enzymes

Carotenoids were determined as previously reported (Lichtenthaler & Wellburn, 1983)**.** GSH was extracted and measured using the method described by (Tanaka *et al*. 1985), which involved grinding 200 mg of plant tissue in 8 ml of a solution of ethylenediaminetetraacetic acid EDTA (0.02 M) at 4°C. The homogenate was deproteinized by adding 0.2 ml of a 0.25% solution of sulfosalicylic acid

105 (SSA). The mixture was centrifuged at 1000 rpm for 5 minutes; then, 1 ml of tris-EDTA buffer (0.02
106 M EDTA, pH 9.6) was added to the supernatant, followed by 0.01 M DTNB (5,5'dithio-bis-2-
107 nitrobenzoic acid). The absorbance was measured at 412 nm.

108 *2.4.2 Enzymatic antioxidants*

109 The antioxidant enzyme activities of ascorbate peroxidase (APX), glutathione peroxidase (GPX),
110 catalase (CAT) and glutathione S-transferase (GST) were assayed in potato tubers. GST activity was
111 measured in potato tubers using the method described by (Habig *et al*. 1974). 500 mg of plant tissue
112 was ground in 5 ml of TBS (Tris-Buffered Saline) buffer solution (50 mM Tris, 150 mM NaCl, pH
113 7.4) at 4°C. Each sample was homogenized with 830 µl of phosphate buffer (0.1 M, pH 6.5), 50 µl
114 of CDNB (1, chloro, 2,4 di nitro benzene) (0.02 M), and 100 µl of GSH (0.1 M). Enzyme activity
115 was measured spectrophotometrically at 340 nm for 1 minute and 5 minutes against a blank that
116 contained distilled water instead of extract.

117 The procedure for obtaining the enzymatic extract from potato tubers was conducted as per (Loggini
118 *et al*. 1999). One gram of plant tissue was homogenized in a phosphate buffer (50 mM, NaK, pH 7.2)
119 at 4°C. The homogenate was subjected to cold centrifugation at 12000xg for 20 minutes. The
120 supernatant acquired was utilized as an enzymatic extract to assess the CAT, APX, and GPX activity.
121 All enzymatic activities were quantified spectrophotometrically.

122 The APX activity was assessed following the protocol established by (Nakano and Asada, 1981),
123 which involves a reaction volume comprising the enzymatic extract, NaK-Ascorbate phosphate buffer
124 (50 mM NaK, 0.5 mM ascorbate, pH 7.2), and hydrogen peroxide (H_2O_2). Absorbance was measured
125 at 290 nm after two minutes. The GPX activity was quantified using the methodology outlined in
126 (Fielding and Hall, 1978) at an absorbance wavelength of 470 nm, with an extinction coefficient (Ɛ)
127 of 2470 M^{-1} cm^{-1}. The CAT activity was conducted following the methodology outlined in (Cakmak
128 and Horst, 1991). The activity of catalase (CAT) was quantified by measuring the reduction in
129 absorbance at 240 nm over three minutes after the addition of H_2O_2 and was reported in nmol min^{-1}
130 mg^{-1} of protein.

2.5 Statistical analysis

The quantitative data are represented using box plots, illustrating the minimum, maximum, median, and mean values. The Kruskal-Wallis and Dunn tests were employed for pairwise comparisons between variables and the control group. Bivariate analysis was employed to better understand the relationship between the examined parameters, and given the data's multivariate nature in this study, principal component analysis (PCA) was more effectively utilized to yield significant results. Statistical analyses were conducted using R statistical software for Windows (Version 3.6.1), with $p<0.05$ deemed significant (Ihaka and Gentleman, 1996).

3. Results and discussion

3.1. Physicochemical parameters and heavy metals

The analyzed sewage sludge exhibits noteworthy agronomic properties (Table 1). Its alkaline pH, along with a high organic matter content, contributes to improved soil structure, water retention, and biological activity. Furthermore, its elevated levels of essential nutrients such as N, P, K, Ca^{2+}, and Mg^{2+} highlight its fertilizing potential. The variability of the physicochemical and biological properties of SS is contingent upon the composition of the wastewater and the treatment methods utilized. (Sharma *et al*. 2006). Moreover, the metal concentrations remain within regulatory limits, supporting the potential for safe agricultural valorization. The concentration of heavy metals in SS varies based on its source (Vieira *et al*. 2014).

Table 1. Physicochemical properties of wastewater, agricultural soil and sewage sludge

Propreties	**Wastewater**	**Agricultural soil**	**Sewage sludge**	
	Measured values (mg L^{-1})	**Measured values (mg kg^{-1})**	**Measured values (mg kg^{-1})**	**European Norms * (mg kg^{-1})**
pH (unit of pH)	7.97	7.3	8.43	-
Electrical Conductivity (EC) (ms cm^{-1})	1.6	1.1	2.56	-
Organic Matter (OM %)	-	2.75	69	-
Magnesium (Mg^{++})	23	390	14052	-
Calcium (Ca^{++})	66	1072	42881	-
Total Nitrogen (N)	74	10.4	5000	-
Phosphorus (P)	13	26.4	7000	-
Potassium (K)	27	160	13000	-

Iron (Fe)	3.77	44.8	462	-
Cooper (Cu)	2.36	18	218	1000
Nickel (Ni)	0.52	09	33	300
Arsenic (As)	-	3.3	8.2	-
Zinc (Zn)	12	27	1800	2500
Cobalt Co	0.04	0.9	2.6	-
Cadmium (Cd)	0.16	0.4	3.7	20
Lead (Pb)	1.4	4.3	119	750
Chromium (Cr)	0.47	12	35	1000
Sulfur (S)	-	17	1221	-
Manganese (Mn)	0.87	10	90	-

The concentrations of heavy metals measured in the roots and tubers of potato (*Solanum tuberosum*) (Table 2) reveal a differentiated distribution of elements, with a preferential accumulation in the roots. The presence of copper (2.2 mg kg^{-1}) in the tubers remains below the European regulatory threshold (10 mg kg^{-1}), indicating a controlled accumulation. The low accumulation of essential nutrients such as Zn, Fe, and Mn in the tubers suggests efficient and regulated uptake. Lead (Pb), cadmium (Cd), and arsenic (As) were found in low concentrations in the tubers (0.024; 0.052 and 0.03 mg kg^{-1} respectively), remaining below the FAO/WHO safety limits of 0.1 mg kg^{-1}.

Regarding non-essential elements such as nickel (Ni), chromium (Cr), and cobalt (Co), their accumulation was mainly restricted to the roots, indicating the presence of an active and effective root barrier. The low levels observed in the tubers can be attributed not only to the physiological mechanisms of the potato plant but also to the favorable agronomic characteristics of the applied sludge. This distribution pattern illustrates a physiological strategy aimed at limiting the translocation of metals to storage organs, thereby protecting the sanitary quality of the tubers (Coelho *et al*. 2025). Such root confinement and reserve organ protection mechanisms explain the plant's low accumulation behavior, as confirmed by several studies (Shi *et al*. 2022) and (Setiyo *et al*. 2020). The concentrations of heavy metals in the tubers were below the food safety limits set by FAO/WHO. They were also lower than the trace element levels found in potatoes harvested from agricultural fields in the industrial area of Jhenaidah, Bangladesh (Islam *et al*. 2018).

Table 2. Metal concentrations (mg kg^{-1} fresh weight) in Roots and Tubers of *Solanum tuberosum* L.

Metals	Fe	Cu	Ni	As	Zn	Co	Cd	Pb	Cr	S	Mn
Roots	42	09	2.55	0.41	58	0.26	0.6	0.8	03	47	18
Tubers	0.4	2.2	0.3	0.03	15.2	0.04	0.052	0.024	0.061	6.7	05
FAO/OMS	0.4	_	_	0.1	20	_	0.1	0.1	0.1	_	_

3.2 Physiological and biochemical responses of potato

The application of SS for fertilization significantly influenced the levels of photosynthetic pigments, as evidenced by the measurements of chlorophyll a, b, total chlorophyll, and total carotenoids in potato leaf tissue (Figure 1). SS markedly ($P < 0.05$) reduced levels of Chl a, b, and Chl t. Chl t levels were 19.76 ± 0.104, 21.17 ± 0.035, and 17.69 ± 0.167 mg g^{-1} FW in treatments T0, T1, and T2, respectively (Figure 1c).

Conversely, the application of SS to potatoes led to a notable enhancement ($P < 0.05$) in carotenoid concentration T2 (5.02±0.058 mg g^{-1} FW) relative to chemical fertilizer (NPK) T1 (4.05±0.051 mg g^{-1} FW) and the control group T0 (2.59±0.37 mg g^{-1} FW) (Figure 1d). Photosynthetic functions are systematically influenced, either directly or indirectly, by heavy metals (Clijsters, 1985), as the application of SS elevates both the heavy metal concentration in the soil and their absorption and accumulation in plant tissues (Lakhdar *et al*. 2009). The decrease in chlorophyll can be ascribed to the disruption caused by heavy metals in the substrate during chlorophyll synthesis, (Singh and Agrawal, 2010). A comparable outcome was also documented by (Singh and Agrawal, 2007) in Palak (*Beta vulgaris*) grown in soil amended with 20% and 40% sewage sludge. Conversely, Lanthanum (La) and cerium (Ce) induced stresses led to a significant increase in pigment content (total chlorophyll and carotenoids) in *Helianthus annuus* plants treated with all concentrations (1_10 µM). (Dridi *et al*. 2022), also, (Belhaj *et al*. 2016) observed an elevation in chlorophyll levels in sunflower (*Helianthus annuus*) plants cultivated in SS. The trend in carotenoid variation was inversely related to that of chlorophyll. Carotenoids, which are photosynthetic pigments, function as non-enzymatic antioxidants that are essential for safeguarding chlorophyll pigment from stress (Halliwell, 1987).

The elevation of carotenoids can be attributed to the plant's defensive mechanism to mitigate stress induced by heavy metals (Singh and Agrawal, 2009). (Singh and Sinha, 2005) have similarly noted a trend in (*Brassica Juncea* L.) cultivated in soil enriched with tannery waste. Prior research has indicated an increase (Zengin, 2013), a decrease (Agrawal and Mishra, 2009), or no alteration (Mishra *et al*. 2006) in the carotenoid content of plants when subjected to heavy metal stress.

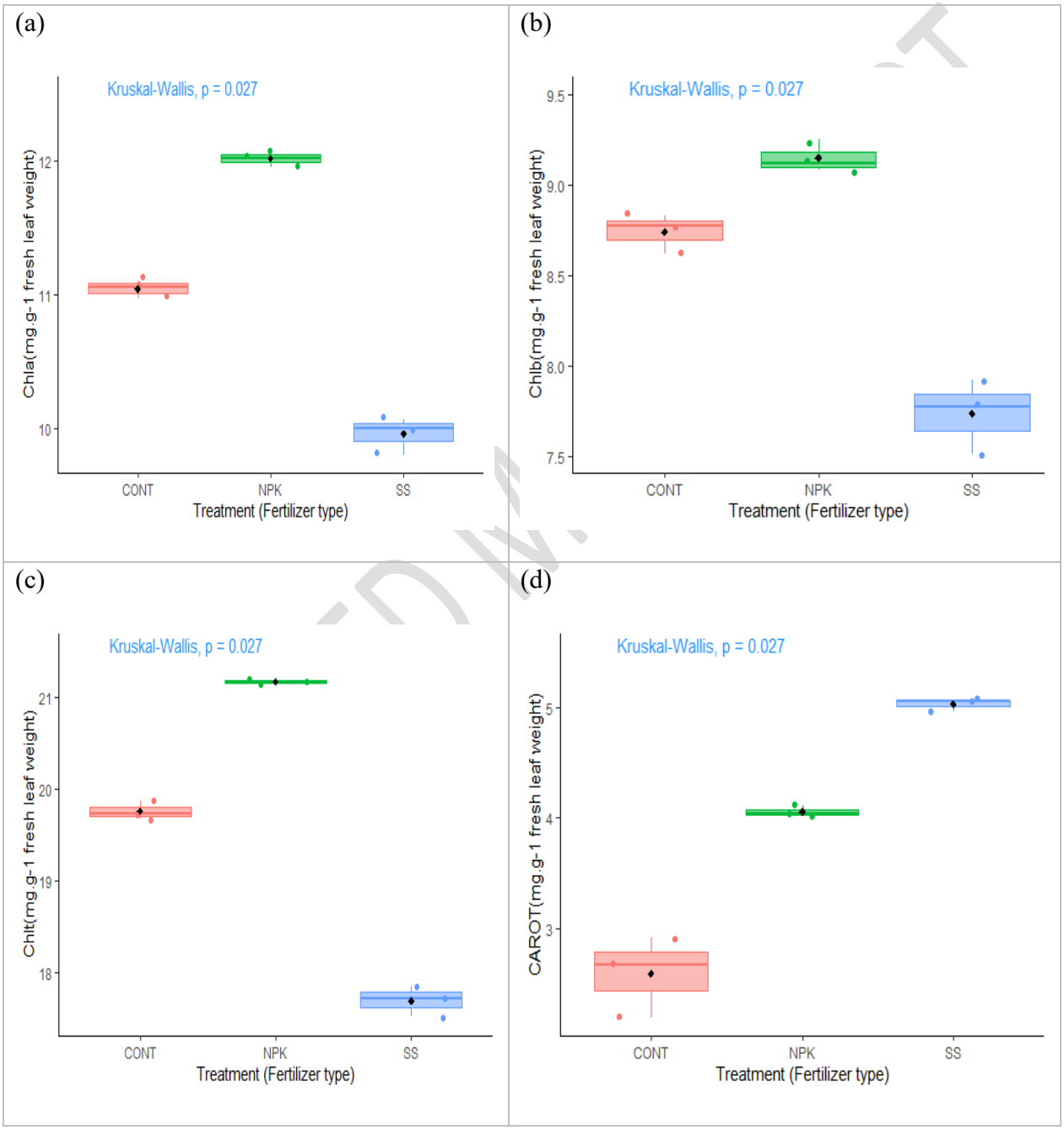


Figure 1. Effect of SS on Chl a (a), b (b), total chlorophyll (c) and carotenoid (d) levels in fresh potato leaves (mg g^{-1} FW).

Proteins are among the most critical elements in any response to heightened cellular oxidation, including enzymes, structural proteins, signal transduction components, ion channels, transporters, transcription factors, and other varieties (Droge, 2002). Figure 2 illustrates a significant reduction in total protein content ($P < 0.05$) in tubers fertilized with SS T2 (1.42±0.21 mg g^{-1} FW) and NPK T1 (1.7±0.1 mg g^{-1} FW) in comparison to the control T0 (2.2±0.2 mg $g^{-}1$ FW) (Figure 2). The measurement of protein levels is a reliable indicator of the plant's overall physiological condition. Consequently, protein content may be influenced by heavy metals for two reasons: the inhibition of specific enzyme activities and the inactivation of proteins containing thiol groups (Favier, 2003) or the heightened activity of proteases leading to the hydrolysis of soluble proteins (Gupta *et al*. 2015). Contrary to our findings, (Singh and Agrawal, 2007) indicated an elevation in protein content in *Beta vulgaris* grown in soil amended with 20% and 40% sewage sludge. Nonetheless, no alteration was observed in sunflower (*Helianthus annuus*) plants cultivated in SS (Belhaj *et al*. 2016).

MDA levels were elevated in tubers treated with SS T2 ($0.7.10^{-3}$±$2.03.10^{-4}$ nmol mg^{-1} FW) and NPK T1 ($0.53.10^{-3}$±$6.23.10^{-5}$ nmol mg^{-1} FW), exhibiting a statistically significant difference ($P = 0.05$) relative to the control T0 ($0.3.10^{-3}$±$2.7.10^{-5}$ nmol mg^{-1} FW) (Figure 3). This significant increase in tubers suggests an intensification of lipid peroxidation, triggered by the presence of heavy metals. Although their concentrations are moderate, their mere presence is enough to disrupt cellular redox homeostasis, underlining the potato's sensitivity to low metal pressures. These metals indirectly catalyze the formation of ROS, leading to oxidative damage to membranes, of which MDA is an end product. This elevation is therefore a direct marker of the oxidative stress generated in the storage organs. Consistent with our findings, MDA levels showed a marked increase in radish plants cultivated on various SLASH mixture ratios at both 45 and 65 days after sowing. (Sharma and Singh 2019), additional researchers have demonstrated an elevation in tissue MDA following exposure to cadmium (Cd) (Corticeiro *et al*. 2006), and sludge applications (Singh and Agrawal, 2007, 2009, 2010).

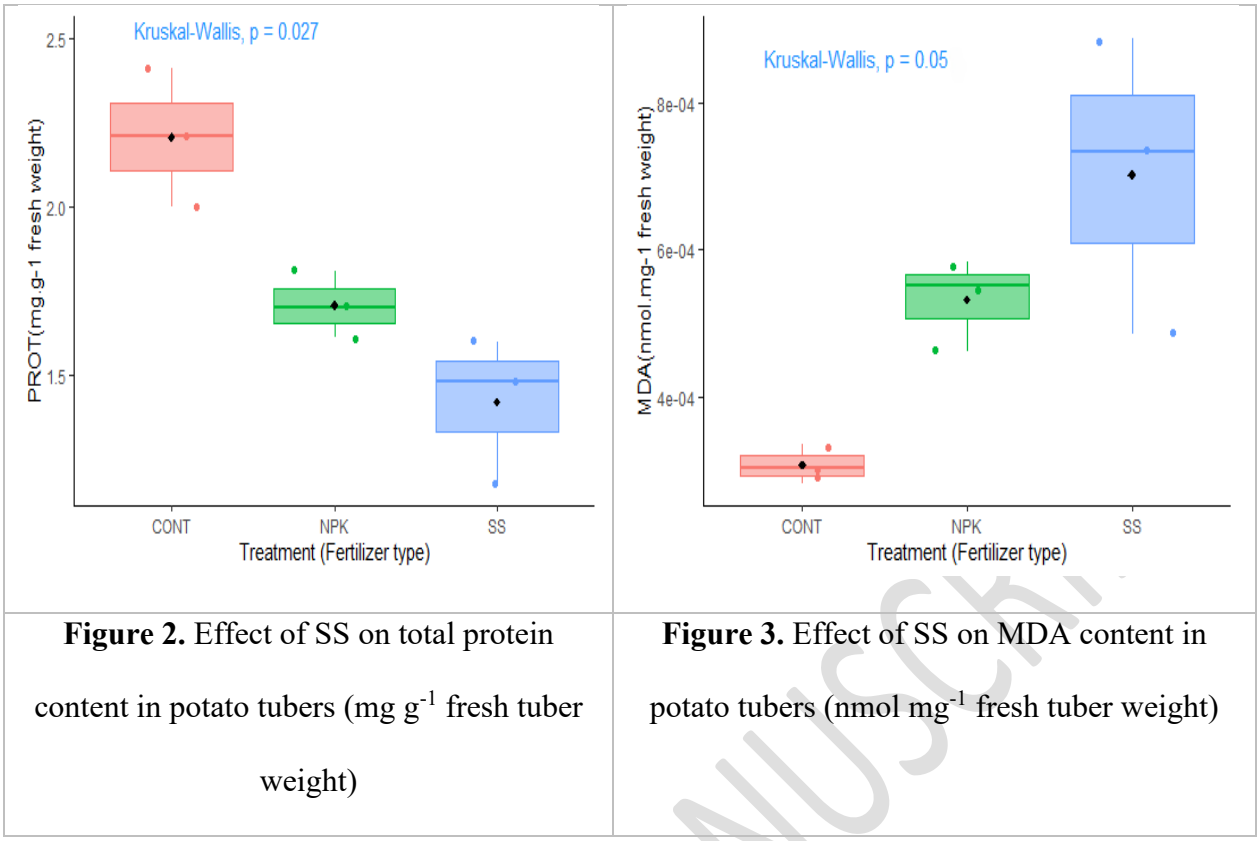


Figure 2. Effect of SS on total protein content in potato tubers (mg g^{-1} fresh tuber weight)

Figure 3. Effect of SS on MDA content in potato tubers (nmol mg^{-1} fresh tuber weight)

The H_2O_2 levels demonstrated a significant elevation ($P < 0.05$) in tubers treated with SS T2 (0.5±0.044 nmol mg^{-1} FW), alongside a modest increase in tubers treated with NPK T1 (0.41±0.03 nmol mg^{-1} FW), albeit less pronounced than that of SS, in comparison to the T0 control group (0.23±0.046 nmol mg^{-1} FW) (Figure 4). Hydrogen peroxide (H_2O_2) is effectively decomposed by the enzymes catalase and peroxidase (PX) (Wu and Tiedemann, 2002). The increased levels of H_2O_2 in tubers even at relatively low metal concentrations, can be attributed to systemic oxidative stress induced by metal accumulation in the roots. H_2O_2 functions as a signal molecule, activating antioxidant defense mechanisms to protect sensitive storage tissues, even in the absence of strong direct contamination. This increase can also be attributed to the activity of superoxide dismutase (SOD), which was not evaluated in this study, an integral component of the antioxidant system in plants, as it converts two $O^{\bullet}_2^-$ radicals into H_2O_2 and O_2, serving as the primary line of defense (Alscher1 *et al*. 2002). According to our findings *Abelmoschus esculentus* plants exhibited a substantial accumulation of H_2O_2 under Aluminum (Al) and Barium (Ba) stress with varying concentrations (0-600 µM), with a more notable effect in aerial parts (shoots and fruits) compared to

roots. (Kouki *et al*. 2024).

The influence of SS and NPK chemical fertilizers on potatoes was evidenced by notable alterations in the non-enzymatic (carotenoids and GSH) and enzymatic (CAT, APX, GPX, and GST) antioxidant defense mechanisms. The collaborative function of these antioxidants is essential for the removal of ROS and the preservation of the physiological redox balance in organisms (Cho and Seo, 2005). GSH serves as a substrate for GPX and GST, which are integral to the removal of ROS. (Noctor *et al*. 2002). GSH is integral to the antioxidant defense system of plants, facilitating the regeneration of ascorbic acid (AsA) through the Asa-GSH pathway (Foyer and Halliwell, 1976). Exposure to heavy metals yields two distinct effects. In certain instances, a significant reduction in GSH is noted, frequently exhibiting a strong positive correlation with the inhibition of antioxidant enzyme activity, whereas in other cases, an increase in GSH levels is observed, as demonstrated in this study.

Figure 6 illustrates that a substantial quantity ($P < 0.05$) of GSH was accumulated in tubers following fertilization with SS T2 ($1.48.10^{-4}\pm1.87.10^{-5}$ µg mg^{-1} protein) and NPK T1 ($8.69.10^{-5}\pm5.55.10^{-6}$ µg mg^{-1} protein), in comparison to T0 controls ($3.33.10^{-5}\pm8.42.10^{-6}$ µg mg^{-1} protein) (Figure 5). Plants have exhibited induction of glutathione synthesis in response to heavy metals (Yadav, 2010). The results demonstrated alignment with the findings of (Belhaj *et al*. 2016) regarding sunflower plants cultivated in SS. Exposure of *Abelmoschus esculentus* to increasing concentrations of Al and Ba resulted in organ-specific changes in GSH content. Elevated levels were found in roots and fruits, while shoots exhibited a marked decline, suggesting an adaptive antioxidative response in reproductive organs. (Kouki *et al*. 2024).

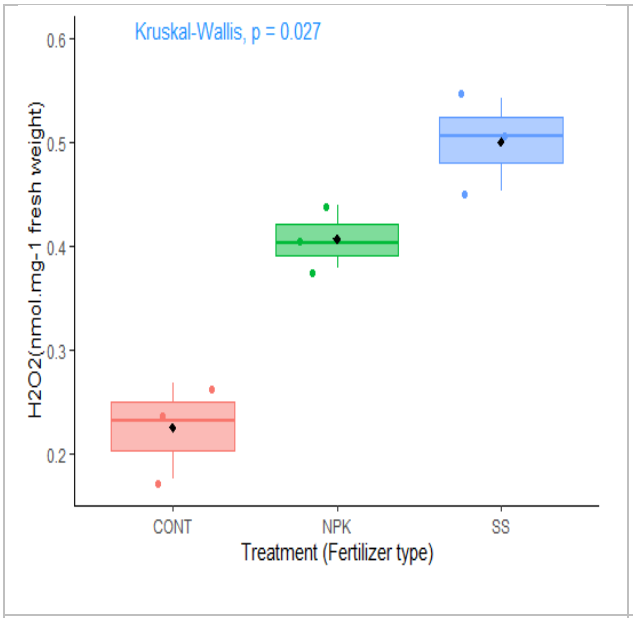


Figure 4. Effect of SS on H_2O_2 content in potato tubers (nmol mg^{-1} fresh tuber weight)

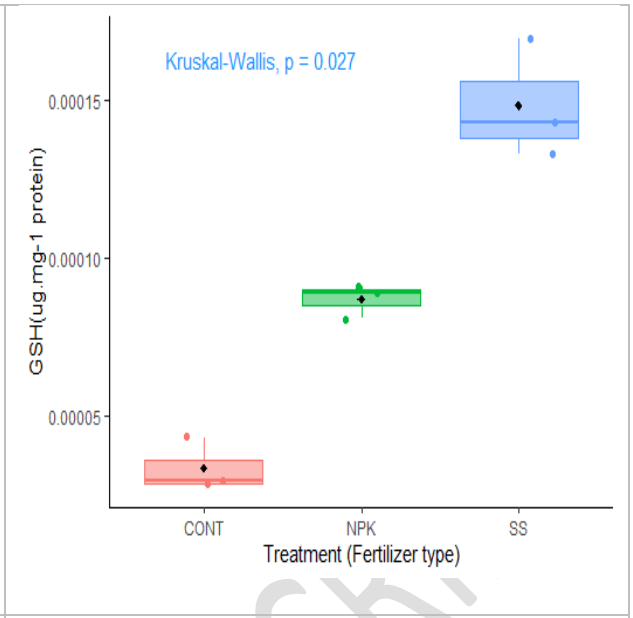


Figure 5. Effect of SS on GSH content in potato tubers (µg mg^{-1} protein)

The activation of antioxidant enzymes in potato tubers is a defense mechanism against oxidative stress. Although metal concentrations in tubers are low, their accumulation in the roots can induce systemic stress. These metals stimulate the production of ROS, such as H_2O_2, A defensive strategy employed by the plant stimulating the activity of enzymes APX, GPX, CAT and GST in order to ensure the maintenance of cell balance and to limit the toxic effects on storage tissues. The regulation of antioxidant enzyme activities is a rapid and efficient mechanism to mitigate excess ROS. (Apel and Hirt, 2004).

Potato tubers exhibited a non-significant ($P > 0.05$) elevation in CAT activity due to T2 SS ($6.85.10^{-8}\pm1.59.10^{-8}$ nmol min^{-1} mg^{-1} protein) in comparison to the other plant groups, T1 ($2.6.10^{-8}\pm1.26.10^{-8}$ nmol min^{-1} mg^{-1} protein) and T0 ($2.04.10^{-8}\pm8.09.10^{-9}$ nmol min^{-1} mg^{-1} protein) (Figure 6). The implementation of SS had minimal impact on catalase (CAT) activity, which was demonstrated to be less active than the other enzymes. The sensitivity of CAT activity to various toxic substances, such as azide, mercaptoethanol, cyanide, 3-amino-1,2,4-triazole, hydroxylamine, urea, and H_2O_2, which have been shown to inhibit CAT, may justify this observation (Bartosz, 1997). The variable response of catalase activity has been noted under metal stress. *Thlaspi* exhibited heightened CAT activity

following treatment with Ni (Freeman *et al*. 2004). The study by (Heidari and Sarani, 2011) indicated that at elevated concentrations of heavy metals, CAT activity was greater when exposed to cadmium (Cd) compared to lead (Pb).

We observed a substantial increase in APX activity ($P<0.05$). The elevation was more significant in tubers treated with T2 SS ($1.16.10^{-6}\pm2.59.10^{-7}$ nmol min^{-1} mg^{-1} protein), indicating greater oxidative stress than that recorded in NPK-fertilized T1 tubers ($5.87.10^{-7}\pm1.58.10^{-7}$ nmol min^{-1} mg^{-1} protein) in comparison to T0 control tubers ($2.81.10^{-7}\pm2.82.10^{-7}$ nmol min^{-1} mg^{-1} protein) (Figure 7). Likewise, (Chou *et al*. 2012) observed that APX activity was elevated under Cd treatment in rice seedlings, while Ni treatment significantly suppressed APX activity in maize roots (Gajewska and Skłodowska, 2005).

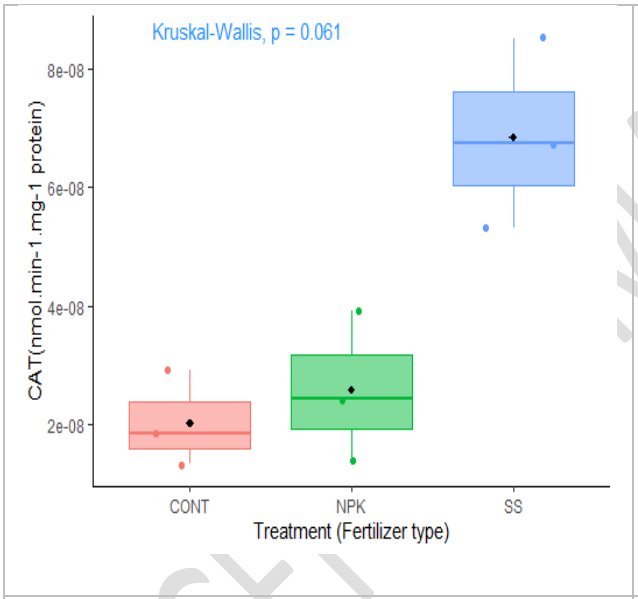


Figure 6. Effect of SS on CAT content in potato tubers (nmol min^{-1} mg^{-1} protein)

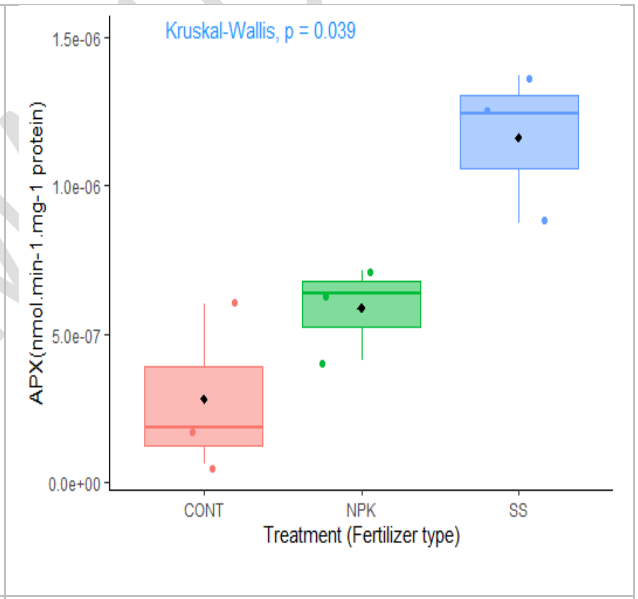


Figure 7. Effect of SS on APX content in potato tubers (nmol min^{-1} mg^{-1} protein)

Glutathione peroxidases (GPXs) constitute a substantial family of varied isoenzymes that utilize GSH to reduce H_2O_2 and organic and lipid hydroperoxides, thereby aiding plant cells in mitigating oxidative stress. GPX activity significantly differs based on plant species and stress conditions (Gill and Tuteja, 2010).

SS T2 ($3.84.10^{-6}\pm5.7.10^{-7}$ nmol min^{-1} mg^{-1} protein) and, notably, chemical fertilizer NPK T1 ($1.63.10^{-}$

^{6}±3.42.10^{-7} nmol min^{-1} mg^{-1} protein) elicited a significant enhancement ($P < 0.05$) in GPX activity relative to the T0 control (6.03.10^{-7}±7.78.10^{-8} nmol min^{-1} mg^{-1} protein) (Figure 8). Elevated GPX activity may indicate significant oxidative stress or a proficient stress response mechanism (Zlatev *et al*. 2006). Stress elevates GPX activity in *C. annuum* cultivars (León *et al*. 2002) but diminishes it in roots and induces no significant alteration in the leaves of *P. sativum* plants subjected to Cd (Dixit *et al*. 2001).

The activity of GST, a primary detoxification enzyme, significantly increased ($P < 0.05$) under T2 SS (0.00086±1.76.10^{-4} nmol min^{-1} mg^{-1} protein) and notably under NPK chemical fertilizer T1 (0.0003±1.88.10^{-4} nmol min^{-1} mg^{-1} protein) in comparison to control T0 (0.0001±1.86.10^{-5} nmol min^{-1} mg^{-1} protein) (Figure 9). GSTs are recognized for their involvement in herbicide detoxification, hormone homeostasis, vacuolar sequestration of anthocyanins, tyrosine metabolism, hydroperoxide detoxification, apoptosis regulation, and plant responses to biotic and abiotic stresses (Dixon *et al*. 2010). (Noctor *et al*. 2002) have indicated that GSTs possess the capacity to eliminate cytotoxic or genotoxic substances that may interact with or harm DNA, RNA, and proteins. Indeed, GST can diminish peroxides through the assistance of GSH, thereby generating scavengers for cytotoxic and genotoxic substances. Other studies indicated a significant increase in GST activity following exposure to various pollutants, including polycyclic aromatic hydrocarbons (PAHs) (Pašková *et al*. 2006), hexachlorobenzene (Roy *et al*. 1995), and atrazine (Tang *et al*. 1998), as well as in the detoxification of heavy metals. (Hamoutene *et al*. 1996), (Ranvier *et al*. 2000), (Ferrat *et al*. 2003).

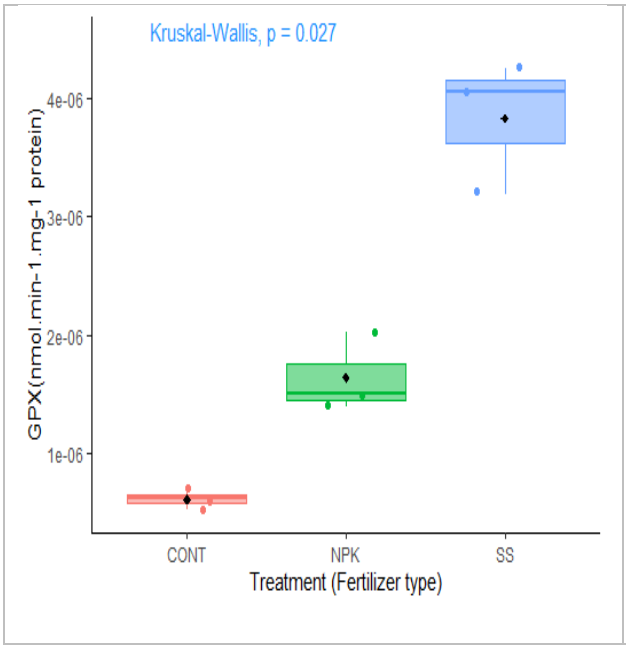


Figure 8. Effect of SS on GPX content in potato tubers (nmol min^{-1} mg^{-1} protein)

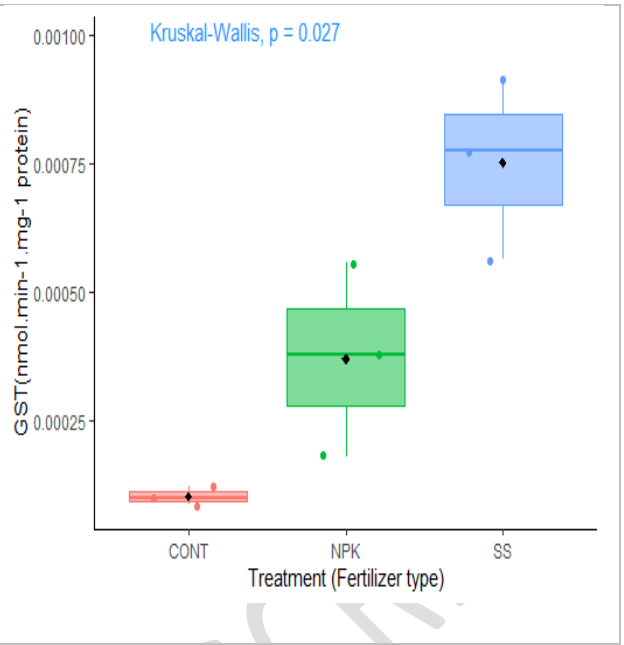
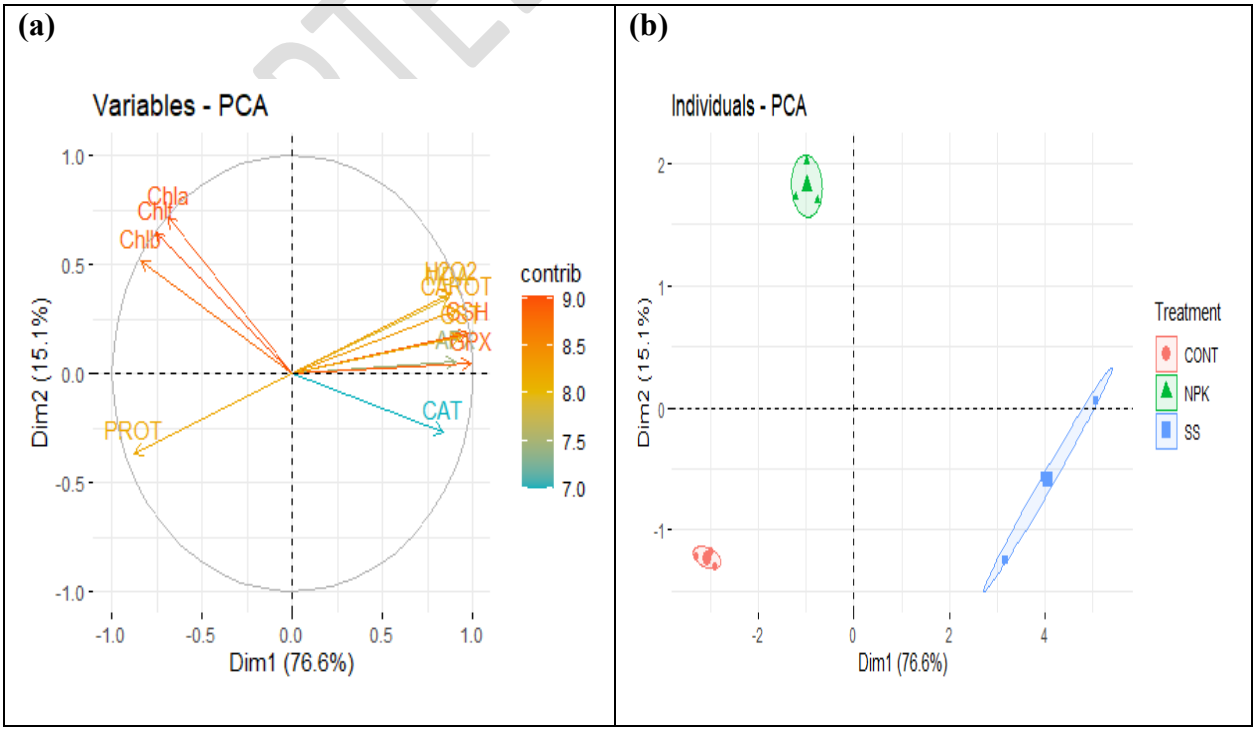


Figure 9. Effect of SS on GST content in potato tubers (nmol min^{-1} mg^{-1} protein)

311

312 The initial two axes of the principal component analysis account for 91.7% of the total variation
313 among the twelve variables under examination, with axis 1 explaining 76.6% of the variation,
314 characterized by high inertia, and axis 2 accounting for 15.1%, characterized by low inertia (Figure
315 10a).



316

317 **Figure 10.** Variable contribution and correlation circle from PCA 1-2 (a). Factorial plane 1-2 used
318 to project treatment groups (b).

319 The results indicate robust positive correlations for MDA, H_2O_2, carotenoids, GSH, GST, APX, GPX,
320 and CAT, alongside significant negative correlations for Chl (a, b, and t) and CAT with the first axis
321 (Dim1) of the PCA, which accounted for 76.6% of the variability independently. The initial
322 dimension distinctly differentiates the impact of SS (T2) (positive) from chemical fertilizer NPK (T1)
323 and the control (T0) (negative). SS is distinguished by its capacity to stimulate the enzymatic
324 activities of APX, GPX, GST, and CAT, enhance GSH synthesis, and elevate levels of MDA, H_2O_2,
325 and carotenoids (Figure 10b). Dim2 accounts for 15.1% of the total variation, indicating a positive
326 correlation with total proteins and chlorophylls (a, b, and t). This axis marginally illustrates two
327 structures demonstrating the impact of NPK chemical fertilizer (T1) and control (T0) on total protein
328 and chlorophyll synthesis (a, b, and t) in comparison to SS.

329 **4. Conclusion**

330 This study aimed to demonstrate that fertilization with sewage sludge (SS) could improve agricultural
331 soil organic matter, nutrient levels (N, P, K, Ca and Mg) electrical conductivity (EC) and heavy metal
332 concentrations. Fertilization with sewage sludge, despite the input of various metals, did not result in
333 excessive accumulation in the tubers of *Solanum tuberosum*. This limited translocation of heavy
334 metals to the tubers is attributable both to the plant's physiological behavior and to the agronomically
335 sound management of the organic amendment, thereby ensuring the food safety of the crop. The
336 mechanisms of root-level confinement and protection of storage organs account for the plant's low
337 accumulator profile, as confirmed by several studies (Shi *et al*. 2022) and (Setiyo *et al*. 2020).

338 Nevertheless, a phytotoxic effect was noted after the application of SS. Potatoes treated with SS
339 experienced oxidative damage, as indicated by elevated MDA levels signifying toxic ROS presence,
340 increased H_2O_2 levels, which also serve as a signaling molecule in plant defense mechanisms, and
341 heightened GSH and carotenoid concentrations reflecting augmented antioxidant capacity. The
342 results indicated that potato (*Solanum tuberosum* L.) has the ability to exhibit various adaptive

responses to oxidative stress. This defensive strategy of plants to mitigate oxidative stress involves modulating their responses to environmental stressors by synthesizing protective compounds (GSH and carotenoids) and activating antioxidant system enzymes (CAT, APX, GPX, and GST). This response pattern underlines the sensitivity of potatoes even to low metal pressures. These mechanisms are essential for cellular protection and plant survival. The findings underscore the significance of physiological and biochemical adaptations for potato resilience in stressful conditions. These findings present promising opportunities for the development of more resilient varieties, providing substantial potential for crop enhancement under adverse conditions.

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