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Low antimony concentration promoted growth, glucosinolate metabolism and antibacterial activity of two *Brassica* species

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Summary

Antimony (Sb), a common rare heavy metal, is found naturally in soils at low concentrations. This study aimed to understand the effect of low Sb concentrations on plant growth and metabolism. To this end, canola (*Brassica napus* L. var. napus) and turnip roots (*Brassica rapa* L. var. rapa) were treated with Sb at low concentrations (8 mg/kg soil) to explore the effect Sb on their growth, photosynthesis, glucosinolate metabolism, redox status, and biological activity. Our results revealed that Sb significantly promoted growth by 1.34 and 1.14-fold in *B. napus* and *B. rapa* compared to control conditions. This increase can be explained by the observed increase in photosynthesis as indicated by increased chlorophyll content. Low Sb concentration significantly improved total glucosinolate accumulation, through improved amino acid production, namely alanine, leucine, isoleucine, valine, methionine, phenylalanine, tyrosine, and tryptophan. There were increases in myrosinase activity, which stimulated glucosinolate hydrolysis to yield health-promoting sulforaphane. In contrast, a low level of ineffective sulforaphane nitrile was detected. Sb also improved antioxidant metabolites (tocopherol) and enzymes (CAT, POX, SOD) in both species but to a bigger extent in *B. napus* by 1.98, 2.77, 2.46, and 2.05 fold, respectively. In conclusion, although high Sb concentrations are toxic, low Sb concentrations can promote biomass and bioactive compound accumulation in *Brassica* spp.

Keywords: *Brassica napus*, *Brassica rapa,* Antimony, antioxidants, glucosinolate and antibacterial activities

Introduction

Heavy metals are naturally present in soils. Some of these heavy metals such as zinc, nickel, and copper in trace amounts are essential for physiological processes as they are required for pigment biosynthesis and enzyme activity (Rout and Das, 2009). One of the rare heavy metals is antimony (Sb). Antimony is present naturally in soils, sedimentary rocks, and water at 0.3-8.6 mg/kg, 0.15-2 mg/kg, and < 1 µg/mL, respectively (Filella et al., 2001; Pierart et al., 2015). Plants predominantly absorb Sb from the soil; however, its effect at low concentrations is not well explored. Zhou et al. (2018) reported that low Sb concentrations increased *Acorus calamus* growth. However, when the Sb concentration in the soil was increased to 250 mg/kg, it resulted in the inhibition of growth. Similarly, low Sb concentration (below 10 mg/L) promoted the germination rate and index of rape seeds (*Brassica napus* L.) and radish (*Raphanus sativus* L.) compared to high Sb concentration (20-100 mg/L), which induce growth inhibition seedlings (Liang et al., 2018).

The US Environmental Protection Agency and the European Union have classified antimony and its derivatives as priority pollutants. The Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal lists Sb as hazardous waste (Liang et al., 2018). Antimony is hazardous to humans at chronic absorption rates of more than 100 mg/day; rats are sensitive to doses ranging from 11 to 75 mg/day. Human exposure to Sb in the workplace is regulated because of its toxicity and possibly carcinogenic characteristics. The limit for Sb in drinking water in the European Union (EU) is 5 µg/L (Filella et al., 2001). The European Commission has established a limit of 0.04 mg Sb/kg for plastic material and objects designed to come into contact with food (EU 2005) (Tschan et al., 2008). Although Sb is a widespread harmful element in the environment, investigations on antimony pollution are relatively limited, particularly in comparison to other well-known toxic elements (Miravet et al., 2005). There are no extensive studies on antimony uptake, transport, and mechanisms regarding toxicity. Consequently, Sb contamination has triggered widespread concern in many regions around the globe. Given the environmental relevance of Sb, a study on its phytotoxic characteristics in plants is crucial since plants are primary producers and can transfer Sb to higher trophic levels. Very few investigations on Sb phytotoxicity and the related bioactive metabolites and their biological activities have been done (Duan et al., 2023). Generally, heavy metal absorption, translocation, and sequestration are critical features of plant life for dealing with heavy metal toxicity. Because heavy metals' physicochemical characteristics are similar to those important microelements, their absorption is accelerated by plasma membrane transporters found in roots (Dalcorso et al., 2013).

Heavy metal accumulation inhibits the electron transport chain, and mitochondrial and chloroplast function, and disrupts the redox equilibrium, eventually leading to production of reactive oxygen species (ROS) (Cuypers et al., 2016). Through a variety of enzymatic and non-enzymatic antioxidant defences, plants can establish defence mechanisms against free radical-induced oxidative stress (Khamis et al., 2019; Abdelgawad et al., 2020; Sofo et al., 2004). Ten mg L^{-1} of antimony enhanced the activity of superoxide dismutase (SOD) enzyme in root cells by 1.94 times and the activities of catalase (CAT) and peroxidase (POX), as well as the levels of antioxidant glutathione (GSH) in root cells by 1.46, 1.38, and 0.52 times, respectively. Moreover, it increased several primary metabolites implicated in four major metabolic pathways: the tricarboxylic acid cycle (TCA cycle), butanoate metabolism, alanine, aspartate, and glutamate metabolism, and alpha-linolenic acid metabolism (Duan et al., 2023).

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The quantitative analysis of plant metabolites identifies the metabolic state of plants (Duan et al., 2023). When these circumstances exist, plants accumulate different types of phytochemicals in plant tissues, besides the antioxidant's arsenal defense, through allocating their primary less active carbohydrate to structural carbohydrates and amino acids to bioactive secondary metabolites. Consequently, the accumulation of these advantageous metabolites improves the nutritious and health-improving benefits of plants (Almuhayawi et al., 2020). For instance, increased amino acid levels increased glucosinolate biosynthesis (Almuhayawi et al., 2020). Glucosinolate accumulation in plants is directly involved in defence against pathogens and herbivores and is broken down into glucose and isothiocyanates by the myrosinase enzyme (Almuhayawi et al., 2021). The hydrolysis of isothiocyanates produced sulforaphane (SF), which is responsible for the high biological activities of several crops particularly *Brassicaceae* species (Björkman et al., 2011; Almuhayawi et al., 2020). Canola (*B. napus* L.) and turnip (*B. rapa* L.) are edible plants that belong to the Brassicaceae family. They are cultivated and consumed for their nutritious and flavourful roots. To the best of our knowledge, there have been no extensive studies on the effect of Sb on the growth, glucosinolate metabolism, and bioactivity of *Brassica* spp.

Therefore, the main goals of this study were first to understand how plants can cope with low Sb concentrations that naturally occur in soil (8 mg/kg soil) and secondly to establish a direct comparison between the response of two edible *Brassica* species canola and turnip to this low Sb level.

Material and methods

Experimental design

Seeds of canola (*B. napus* L. var. napus) and turnip (*B. rapa* var. rapa L.) were surface sterilized in 5% (v/v) sodium hypochlorite for 20 minutes. In 20×25 cm pots, soil potting mix (Tref EGO substrates, Moerdijk, Netherlands) was used. The soil was mixed with $KSb(OH)_6$ to final concentrations of 0 mg/kg (control) and 8 mg/kg soil. The selection of this low concertation is based on its normal concentration in nature as well as a preliminary study evaluated the impact of various natural Sb concentrations (ranging from 0.5 to 11 mg Sb/kg soil) on the growth of *B. napus* and *B. rapa*. In this initial experiment, a growth-enhancing effect was observed at the specific Sb concentration of 8 mg/kg soil. This outcome was subsequently validated in the main experiment.

The pots were then transferred to a controlled growth room (16/8 h day/night photoperiod, light intensity of 250 µmol m^{-2} s^{-1}, 60/70% (w/v) humidity, and 21/18 °C air temperature). The control and treatment pots were watered daily to 60% soil water content. Plants were collected six weeks following the Sb treatment. Shoots of plants of each treatment were washed with distilled water, dried on filter paper, and weighed to for fresh weight (FW) measurements. Shoots of some plants were dried (air oven at 70 °C) for 72 h for measuring dry weight (DW). Fresh shoots were collected and kept at -80 °C for subsequent biochemical analysis. Five biological replicates of each species for each treatment were analysed. Moreover, each biological replicate was obtained by pooling leaves from 5 plants. Soils were also collected at the end of the experiment to determine the antimony content in the soil.

Pigment content evaluation

According to Al Jaouni et al., (2018) pigment concentrations in both brassica cultivars were separated and quantified. Using a MagNALyser (Roche Diagnostics, Vilvoorde, Belgium), pigments were extracted in acetone from the plant shoots (Roche Diagnostics). The mixture was then centrifuged at 14,000× g for 20 minutes at 4 °C, and the supernatant was filtered (Acrodisc GHP filter, 0.45 m 13 mm). The concentrations were determined by high-performance liquid chromatography (HPLC) (Shimadzu SIL10-ADvp, reversed phase). Pigments were separated on a C18 silica column (Waters Spherisorb, 4.59 × 250 mm, 4.5 µm ODS1, at 45 °C), using a mobile phase, as follows: (A) 81:9:10 acetonitrile/methanol/water and solvent; (B) 68:32 methanol/ethyl acetate, at a flow rate of 1.0 mL/min at 25 °C. A diode array detector was used at 420, 440, and 462 nm (Shimadzu SPD-M10Avp) to assess chlorophyll a, chlorophyll b, and carotenoids.

Markers of oxidative stress

To determine lipid peroxidation levels, 50 mg tissues (leaves and well-cleaned root discs) were extracted in 1 mL 80% ethanol using a MagNALyser (Roche Diagnostics). The extract's malondialdehyde (MDA) content was then measured using the thiobarbituric acid test (Hodges et al., 1999). Cayman Chemical's (Ann Arbor, MI) Protein Carbonyl Colorimetric Assay Kit was used to quantify protein carbonyls as oxidative damage indicators (Levine et al., 1994). The Xylenol orange technique, which relies on peroxide-catalyzed oxidation of Fe^{2+}, was used to measure hydrogen peroxide (H_2O_2) in trichloroacetic acid (TCA, 0.1%) extract of plant material (Jiang et al., 1990). To minimize non-specific interactions, each sample was compared to its catalase (CAT) treated counterpart.

Determination of antioxidant metabolites

Total antioxidant capacity (TAC) (ferric reducing antioxidant power, FRAP) was determined in ice-cold 80% ethanol using a MagNALyser (Roche Diagnostics) and quantified using trolox as a reference, as described by Benzie and Strain (1999). Plant materials were extracted in 80% ethanol using a MagNALyser (Roche Diagnostics) for ascorbate (ASC) and GSH determination. HPLC was used to measure ASC and GSH. Fresh plant materials were homogenized in 80% ethanol before centrifugation at 5000 rpm for 15 minutes to extract phenols and flavonoids. Following that, the clear extract was utilized to quantify the phenols and flavonoid contents using the Folin-Ciocalteu and aluminium chloride tests, respectively.

Antioxidant enzyme evaluation

One hundred mg of frozen plant samples were crushed in 1 mL buffer [50 mM potassium phosphate, pH 7.0, 10% (w/v) polyvinylpyrrolidone (PVP), 0.25% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM ASC] using a MagNALyser (Roche Diagnostics). The clear supernatant was used to assess the activities of antioxidant enzymes such as superoxide dismutase (SOD), EC 1.15.1.1), POX (EC 1.11.1), CAT (EC 1.11.1.6), GSH peroxidase (GPX, EC 1.11.1.9), ASC peroxidase (APX, EC 1.11.1.11), GSH reductase (GR) and GSH transferase (GST, EC 2.5.1.18). The enzyme activities were assessed through kinetic reactions conducted in 200 µL volumes at 25 °C, utilizing a micro-plate reader (Synergy Mx, Biotek Instruments Inc., Vermont, USA). SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm, as described by Dhindsa et al. (1982). The oxidation of pyrogallol ($\varepsilon 430 = 2.47\ mM^{-1} \cdot cm^{-1}$) was used to measure POX activity (Kumar and Khan, 1982). The breakdown of H_2O_2 at 240 nm was used to assess CAT activity (Hugo and Lester, 1984). GR activity was assessed by monitoring the absorbance change at 340 nm and then calculated using the 6.22 mM^{-1} cm^{-1} extinction coefficient (Enzyme activity = (OD/time * reaction volume)/(used extract volume * mg protein * extinction coefficient). The measurement of APX, DHAR, and GR activities followed the methodology reported by Khamis et al. (2019). GPX activity was determined by measuring the reduction

in NADPH absorbance at 340 nm (with an ε340 of 6.22 mM^{-1} cm^{-1}, as recommended by Drotar et al. (1985). Tocopherols were extracted in hexane and measured by HPLC (Shimadzu SIL10-ADvp, reverse-phased, at 4 °C) using dimethyl tocol (DMT) as an internal standard (5 ppm) (Abdelgawad et al. 2015(. After drying by using vacuum evaporator (Roche Diagnostics), the extract was reconstituted in hexane, and the separation and quantification of tocopherols were performed using High-Performance Liquid Chromatography (HPLC) on a Shimadzu instrument ('s Hertogenbosch, The Netherlands). The HPLC system employed normal phase conditions with Particle Pack 5 µm column (length 250 mm, i.d. 4.6 mm). For quantification, dimethyl tocol (DMT) was used as an internal standard at a concentration of 5 ppm. The obtained data were then analysed using Shimadzu Class VP 6.14 software.

Amino acid analysis

Amino acids were extracted from 200 mg of each plant sample in 1 mL of 80% (v/v) aqueous ethanol at room temperature (Alotaibi et al., 2021). Norvaline was used as an internal standard to accurately evaluate and correct various mass spectrometry processes. The homogenate was centrifuged at 14,000× g for 30 minutes at 25 °C. The supernatant was transferred to new tubes and then subjected to drying. The resulting pellet was resuspended in 1 mL of chloroform, followed by centrifugation at 14,000× g for 30 minutes at 25 °C. The obtained pellet was mixed with the chloroform-suspended pellet and subjected to another round of centrifugation for 10 minutes at 20,000× g. After this step, 25 µl of aqueous phase was filtered using Millipore microfilters with a pore size of 0.2 µm for amino acid analysis. The amino acids were separated using a BEH amide 2.1 50 column on a Waters Acquity UPLC-tqd system (Milford, MA, USA). A 10 µL sample was injected, and elution was carried out using a gradient of 0.1% formic acid (FA) in H_2O and 0.1% FA in acetonitrile. Throughout the process, the sample was maintained at 20 °C, and the column temperature was set to 30 °C.

Total glucosinolate extraction and determination

Total glucosinolates were extracted and determined by following the method of Almuhayawi et al. (2020). The thio-glucosidase coupled test was validated by measuring glucosinolate levels in different Brassicaceae species and comparing the results to those described in the literature. Fresh samples were steamed for 2 minutes on a perforated tray over boiling water before glucosinolate extraction. This length of steaming was sufficient to inactivate myrosinase activity. A porcelain mortar was used to homogenize 1 g of plant sample in 3 mL of MeOH: water (70:30; v:v) containing trifluoroacetic acid (TFA, 1.5 g/L) (Sigma). The extracts were transferred to stoppered Erlenmeyer flasks and conditioned in a thermostatic bath with continual agitation. This extraction lasted 30 minutes at 70 °C. The collected supernatants were filtered using qualitative filter sheets (Whatman number 1) and then evaporated at 40 °C until complete solvent evaporation. After cooling and centrifugation (at 8000 g for 20 minutes for 72 hours). The resulting dry precipitate was reconstituted in HEPES-KOH (0.2 mM, pH 7.0) in the same container for 10 min for assessing total glucosinolate concentrations, about 10 µL of the extract was mixed with thioglucosidase (myrosinase enzyme, 0.12 U) in HEPES-KOH (0.2 mM, pH 7.0) for 24 hours at 37 °C. To halt the process, an 18 mM perchloric acid solution was added. Control samples were prepared by either replacing the sample extracts with buffer or adding the stopping solution immediately. The amount of glucose generated from glucosinolate hydrolysis by thioglucosidase was calculated using stoichiometry, where 1.0 mol of produced glucose corresponds to 1.0 mol of total glucosinolate. The determination of total glucose was enzymatically performed using a glucose oxidase/peroxidase kit, with sinigrin and allyl-glucosinolate used as calibrants and positive controls, respectively.

Glucosinolate, sulforaphane (SF), and sulforaphane nitrile (SFN) content extraction

Similar to total glucosinolates, fresh samples were steamed for 2 minutes on a perforated tray over boiling water, which demonstrated to give the highest level of glucoraphanin by rapidly inactivating myrosinase activity. Glucoraphanin content was extracted from 0.5 g of steamed samples in 5 mL methanol (70%) and the liquid was then agitated in a swirling water bath for 15 minutes at 70 °C to ensure maximal recovery. The mixture was cooled, filtered (Whatman No. 1 filter paper), and washed with 5 mL of 70% methanol. The methanol fraction was dehydrated for 30 minutes at 50 °C using a rotary evaporator. The residue was redissolved in 5 mL of methanol. The resulting extract was initially introduced to Sep-Pak for measurement of glucoraphanin and progoitrin concentration (Almuhayawi et al., 2020). The eluate was filtered through a nylon filter using a Vac 6 cc cartridge (0.2-um). Twenty µL of the filtrate was injected into a Zorbax Eclipse SB-aq column (150 X 4.6 mm i.d., 5 mL/min) with a mobile phase of acetonitrile: water: formic acid (1:99:0.1v/v/v) with a flow rate of 1 mL/min. We identified glucoraphanin and progoitrin using a DAD detector (235 nm), and the concentration was determined using a standard curve of glucoraphanin and progoitrin concentrations. SF and SFN were extracted from *B. napus and B. rapa* leaves by first crushing the samples into a fine powder using liquid nitrogen and analysed following the method of (Almuhayawi et al., 2020). The mixture was allowed to autolyze at room temperature for 8 hours to achieve complete myrosinase catalysed conversion of glucosinolate. In addition, a test was conducted to convert glucosinolate to SF and SFN using Na-KO4 buffer (pH 7.4, 0.01 M) for 4 hours at 36 °C, yielding highly similar results. Subsequently, NaCl and Na_2SO_4 were added in a 1:0.75:1 ratio (w/w/w). To eliminate any remaining water traces, $MgSO_4$ was introduced and then re-dissolved in 5% acetonitrile in water (v/v) and passed through a 0.22-µm nylon membrane for further processing.

For quantifying SF content, the extract was introduced to an Oasis HLB 3 cc cartridge, and the eluate was purged with N2 and dissolved in 0.5 mL of 1% (v/v) acetic acid. Elution was performed using a mixture of acetonitrile: water (30:70, v/v) at a flow rate of 0.8 mL/min. Detection was carried out using a DAD at 202 nm. To analyse the SFN content, the extracted samples were concentrated and subjected to LC-ESI-MS/MS analysis using a Q-Tof Premier mass spectrometer connected to an Alliance 2695 HPLC system. Separation was performed on an Atlantis T3 C18 column (100 mm × 2.1 mm; 3 µm) maintained at 40 °C. Mass spectral data (positive mode with a mass range of *m/z* 100 to *m/z* 1000), capillary voltage and cone voltage (3 kV and 30 V, respectively) and collision-induced fragmentation (MSe mode, 12 eV to 20 eV energy with helium as the collision gas) were used. The elution was achieved using 10 mM ammonium acetate buffer (pH = 4.5) and 0.1% formic acid in acetonitrile at a flow rate of 0.2 mL/min. Mass spectroscopy were used for analysis. Leucine-enkephalin was used as an internal reference compound.

Antibacterial activity

Using the disc diffusion method, the ethanolic extracts of leaves from each *Brassica* species were evaluated for their antibacterial activity against *Streptococcus* sp., *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. A suspension of each test bacteria (10^6 CFU/mL) was evenly spread on Mueller-Hinton agar. Next, extracts (20 µL/disc) were applied to sterilized filter paper rounded discs with a diameter of 5 mm. As a negative control, ethanol was used. Subsequently, the prepared discs were positioned on the agar plates

and incubated at 37 °C for 24 hours. The inhibitory zones around the discs were measured using a Vernier caliper.

Statistical Analysis

A fully randomized design was used to test treatment effect. The data are displayed using the GraphPad Prism 8.4.2 program and are presented as (means ± standard error). Five biological replicates of each species for each treatment were analysed. To reduce potential variations that might arise from individual differences between plants, each biological replicate was obtained by pooling leaves from 5 plants. One-way ANOVA results were employed for the statistical analysis at $p<0.05$. The relevant control value was compared to each experimental value.

Results and discussion

Herein, we investigated the impact of Sb at its low concentration in soil (8 mg/kg soil) on growth, photosynthetic pigments, antioxidants capacity and glucosinolate metabolism of *B. napus* and *B. rapa*, to test the hypothesis that Sb treatment could stimulate the growth and bioactive metabolites in brassica species. Antimony at moderate and high concentrations has been related to unfavourable plant effects such as growth retardation, decreased biomass and photosynthesis, ROS production, and lipid peroxidation (Liang et al., 2018). The effect of low Sb concentrations on *Brassica* species was not well studied, which will further affect the *Brassica* output and farmers' economic revenue. Therefore, exploring the effect and phytotoxicity of Sb treatment on brassica species is essential.

Antimony stress stimulated the growth of *Brassica* species by increasing photosynthetic pigments

Sb treatment significantly increased the growth of both *B. rapa* and *B. napus* compared to the control. There were signifi ant differences ($p < 0.05$) in growth values between the two species. Whereas the shoot fresh weight and dry weight of *B. rapa* under Sb treatment were 1.14 and 1.31-fold the control treatment, respectively (Fig. 1). In addition, the shoot fresh weight and dry weight of treated *B. napus* was 1.35 and 1.34-fold the control treatment, respectively (Fig. 1). In line with our results, Liang et al., (2018), reported that the rape seed germination was enhanced by a low Sb concentration. According to this investigation, the Sb concentration threshold for radish and rape plants on hydroponic culture was 10 mg/L. Moreover, *Acorus calamus* was grown in soil at different concentrations of Sb 0, 250, 500, 1000, and 2000 mg/kg soil, Sb promoted the growth of *A. calamus* at low concentrations, and by increasing the Sb concentration in soil, the growth was sharply inhibited (Zhou et al., 2018). Sb stress inhibits plant development and biomass accumulation by reducing photosynthesis and affecting the production of soluble sugars and soluble proteins (Duan et al., 2023).

To demonstrate the link between the increase in shoot DW and FW and Sb heavy metal accumulation in tissues. Because heavy metals' physicochemical characteristics are similar to those of important microelements, their absorption is accelerated by plasma membrane transporters found in roots (Dalcorso et al., 2013). Heavy metal concentrations in both brassica species were evaluated. Accordingly, Sb was more accumulated in *B. rapa* (19.11±0.7 µg/g) which represented 1.18-fold the content of Sb in *B. napus* (Fig. 1). In line with our results, Liu et al. (2021) reported that *B. rapa* is a popular green vegetable crop and collects significant levels of heavy metals. Otherwise, *B. napus* accumulate higher Cd and Zn in shoots than in roots, indicating efficient translocation of Cd and Zn into shoots. Flowering Chinese cabbage (*B. rapa*) collects Cd more effectively than Hg or Cr (Rossi et al., 2012). Several studies illustrated that the

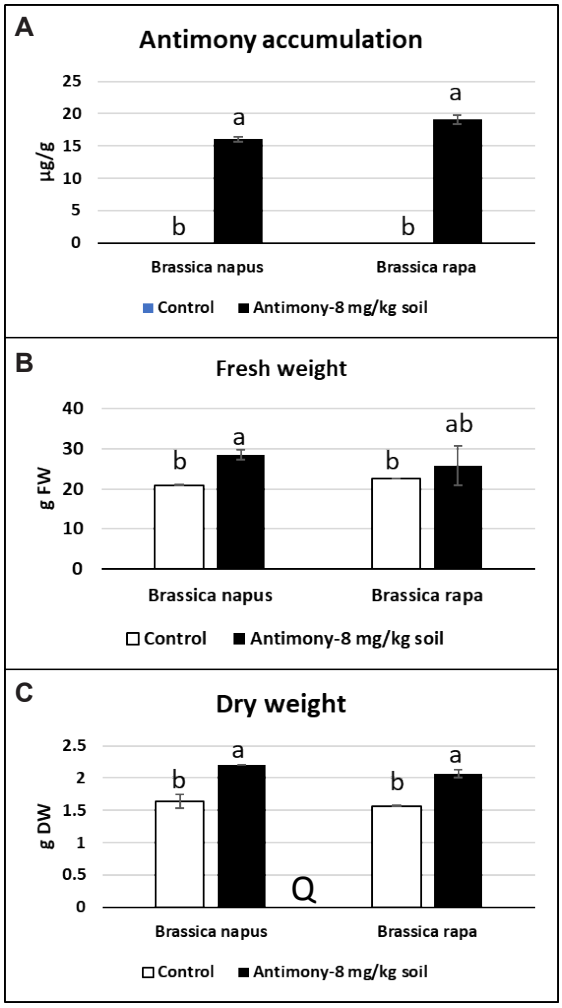

Fig. 1: Antimony accumulation (Sb) and biomass accumulation of *Brassica napus* and *Brassica rapa* under control and Sb (8 mg/kg) treatment conditions. A) Sb content, B) shoot fresh weight (FW), C) shoot dry weights (DW). Data are represented by the means of five biological replicates ± standard error. At $p<0.05$, various small letters (a, b, c…) indicate significant differences between means.

effect of heavy metals on growth parameters was different depending on the plant type. For instance, Sb treatments reduced the growth parameters in rye and wheat. However, rye could collect more Sb than wheat during Sb treatment. In general, the low Sb concentration in soil at 8 mg/kg could promote the growth of both Brassica species, and increasing the Sb concentration will inhibit the growth parameters. Where, the acceptable Sb intervention values in soil and groundwater in the Netherlands are 15 mg/kg and 20 µg/L, respectively (Tschan et al., 2008).

To investigate the impact of the observed increase in Sb accumulation in treated Brassica species. we evaluated the plant's photosynthetic pigments. The increase in growth was followed by increasing in the photosynthesis pigments. Herein, antimony treatments significantly ($p < 0.05$) induced the content of chlorophyll (Ch) a and Chb in treated *B. napus* which represented 1.36 and 1.25-fold the control treatments, and in *B. rapa* which represented 1.2 and 0.75-fold of the corresponding controls, respectively, (Fig. 2). On the other hand, Sb treatments significantly ($p < 0.05$) boosted the contents of carotenoids of both *B. napus* and *B. rapa* by 57 and 61%, respectively, compared to the corresponding controls (Fig. 2). *Brassica rape* represented significantly ($p < 0.05$) higher reduction in chlorophyll b (Chb), but significantly ($p < 0.05$) increased the Cha and Ch a + Ch b

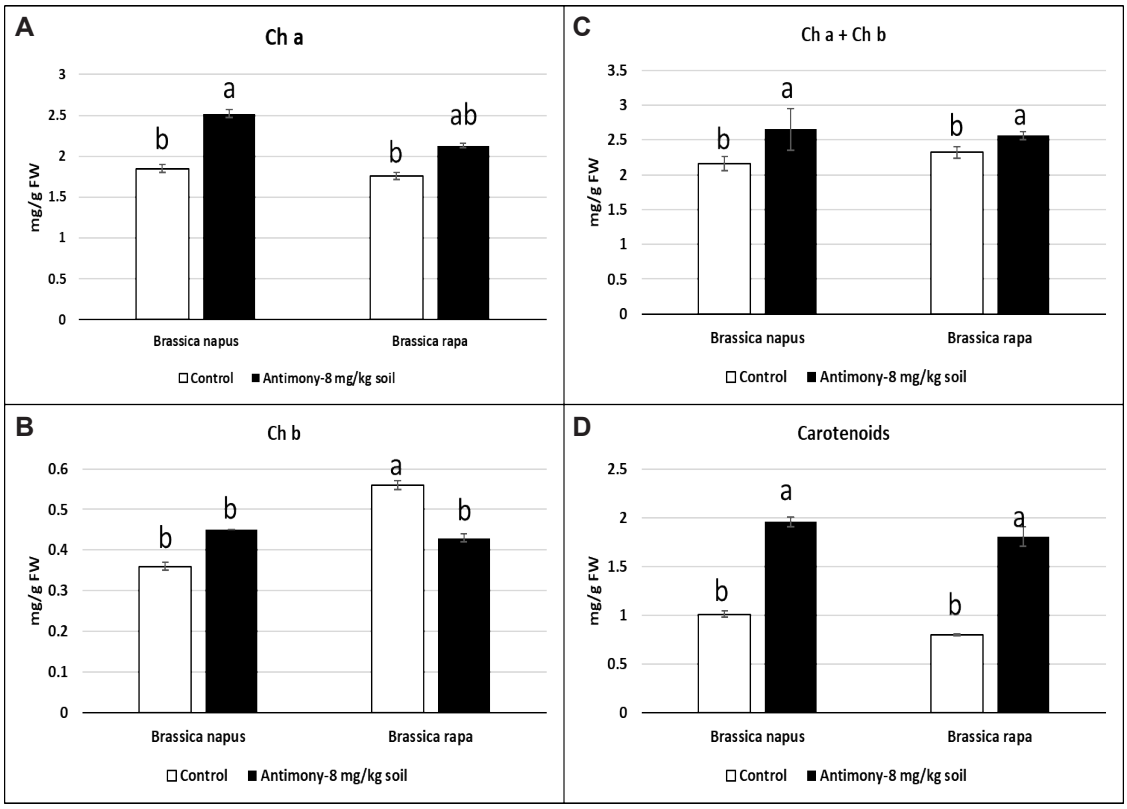

Fig. 2: Pigment contents of *Brassica napus* and *Brassica rapa* under control and Sb (8 mg/kg) treatment conditions. A) Chlorophyll a (ch a), B) Chlorophyll b (ch b), C) Chlorophyll a+b (ch a+ch b), D) carotenoids. Data are represented by the means of five biological replicates ± standard error.

and showed improvement in carotenoids compared to *B*. *napus* under Sb treatments.

Antimony stress enhances the accumulation of antioxidant metabolites

To determine whether Sb accumulation in Brassica tissue can induce ROS generation and cellular damage. Under Sb treatment, the levels of H_2O_2 and MDA were about 1.4 and 1.6-fold at *B*. *napus* and at *B*. *rapa* by 2.3, and 2.2-fold, respectively, compared to the corresponding controls (Tab. 1). Cuypers et al., (2016), reported that under heavy metal stress, the plant-developed priming and accumulation for H_2O_2 in roots may potentially act as a protective mechanism against heavy metal stress. It was hypothesized that H_2O_2 accumulation in the roots of bean seedlings under Zn and Cu stress, and pine seedlings under Cu stress, might represent a protective mechanism via enhanced lignification of the root cell walls, acting as apoplastic trapping for the heavy metals. Mostofa et al. (2020) suggested that Sb stress desynchronized ROS-detoxifying pathways, where the major ROS-detoxifying process in leaves is based on the induction of non-enzymatic antioxidants. In this context, plants use antioxidant arsenal components to combat oxidative stress according to the type of stress and plant developmental stage (Abdelgawad et al., 2020).

Herein, Sb treatments significantly increased the activities of ROS scavenging enzymes in response to the Sb effect in both *B*. *napus* and *B*. *rapa*. The present results revealed that the levels of total antioxidant capacity (TAC), antioxidants metabolites such as GSH, ASC, phenolics, and tocopherols and enzymes (CAT, POX, SOD, APX, GR, and GPX) were significantly increased under Sb treatment compared to the control.

The levels of phenols and flavonoids were significantly ($p < 0.05$) increased under Sb treatment compared to the control and *B*. *napus* showed the highest levels of both phenols and flavonoids compared to *B*. *rapa*. Supportively, phenolic and flavonoid compounds have been identified for their antioxidant effects (Almuhayawi et al., 2020). Due to their high phenolic component concentration, Brassicaceae, particularly *B*. *napus* and *B*. *rapa* could be regarded as a vital nutritious diet and serve as a source of valuable active metabolites. High phenols and flavonoids function as a natural defence against oxidative stress by scavenging free radicals. Thus, the nutritional condition of plants is connected to their content of secondary metabolites and antioxidant chemicals such as phenols (Almuhayawi et al., 2021). Furthermore, the levels of tocopherol under Sb treatments were significantly ($p < 0.05$) triggered in *B*. *napus* and *B*. *rapa* about 1.93 and 1.22-fold, respectively, relative to the corresponding control.

Increasing the activity of antioxidant enzymes was reported in many plant species to relieve the effect of oxidative stress (Cuypers et al., 2016). Additionally, the levels of TAC and SOD in *B*. *napus* under Sb treatments were significantly induced by 2.3, and 2.05-fold, respectively, compared to the corresponding control (Tab. 1). In line with our findings, Duan et al. (2023) reported that rice (*Oryza sativa* L.) grown under 8 mg L^{-1} Sb stress revealed an increase in the activity of the SOD in root cells by 1.94 times, as well as the activities of CAT, POX, and the quantities of the GSH in the root by 1.46, 1.38, and 0.52 times, respectively. Herein, the activity of CAT and POX was induced by about 2.77, and 2.46- fold, respectively, in Sb-treated *B*. *napus*. High levels of POX and CAT were intended to alleviate the H_2O_2 generated by SOD by converting H_2O_2 into H_2O and O_2 (Zhou et al., 2022) or converted to H_2O by GSH (Duan et al., 2023). Moreover, increasing SOD content in maize tissues under heavy metals treatments was considered a protective mechanism, and the elevated GR activity reflects the regeneration of reduced glutathione, which might have been oxidized directly by ROS (Abdelgawad et al., 2020).

Tab. 1: The antioxidant contents in control and Sb (8 mg/kg) treated *Brassica napus* and *Brassica rapa*. Data are represented by the means of five biological replicates ± standard error.

Antioxidants	*Brassica napus*		*Brassica rapa*	
	Control	Sb 8 mg/kg soil	Control	Sb 8 mg/kg soil
Oxidative marakers (µmol/gFW)				
Malondialdehyde (MDA)	8.97±0.77^{c}	14.21±0.3^{b}	10.87±0.27^{c}	24.24±0.37^{a}
Hydrogen peroxide (H_2O_2)	199.8±18.4^{c}	278.3±7.2^{b}	248.4±4.8^{c}	568.5±8.6^{a}
Antioxidant metabolites (mg/gFW)				
Total antioxidant capacity (TAC)	11.16±0.18^{c}	26.1±0.29^{a}	9.1±0.9^{c}	13.35±0.29^{b}
Phenol	4.11±0.15^{b}	8.62±0.27^{a}	3.46±0.22^{b}	4.42±0.19^{b}
Flavonoids	2.69±0.02^{c}	6.35±0.06^{a}	1.88±0.22^{c}	3.34±0.04^{b}
Glutathione (GSH)	0.35±0^{c}	0.66±0^{a}	0.3±0.02^{c}	0.45±0.01^{b}
Ascorbic acid (ASC)	1.72±0.05^{b}	3.54±0.09^{a}	1.46±0.09^{b}	3.83±0.07^{a}
Tocopherol	4.42±0.03	8.54±0.2	3.38±0.29	4.13±0.18
Antioxidant enzyme activities (µmol/mg protein·min)				
Peroxidase (POX)	2.13±0.02^{b}	5.91±0.1^{a}	1.94±0.29^{b}	4.76±0^{a}
Catalase (CAT)	13.15±0.89^{c}	32.4±0.17^{a}	10.19±1.51	21.38±0.06^{b}
Superoxide dismutase (SOD)	303±4.8^{c}	622±5.1^{a}	257±22^{c}	587±4^{b}
Ascorbate peroxidase (APX)	0.47±0.01^{b}	1.64±0.07^{a}	0.48±0.09^{b}	1.45±0.01^{a}
Diphenyl picrylhydrazyl (DPPH)	0.39±0.01^{a}	0.40±0.01^{a}	0.32±0.04^{a}	0.37±0.01^{a}
glutathione reductase (GR)	0.38±0.01^{b}	1.15±0^{a}	0.29±0.06^{c}	1.07±0.02^{a}
Glutathione peroxidase (GPX)	0.7±0.03^{c}	1.05±0.05^{a}	0.48±0.04^{d}	0.65±0.01^{b}

At $p<0.05$, various small letters (a, b, c...) within a row indicate significant differences between means.

Exposure to Sb induced amino acids precursors derived glucosinolate accumulation

Brassica is rich in antioxidants, vitamins, minerals, glucosinolate and hydrolysis products (Matusheski Juvik and Jeffery, 2004). Free amino acids are precursors for glucosinolates, aliphatic glucosinolates are derived from methionine, leucine, alanine, isoleucine, or valine, but aromatic glucosinolate is built from phenylalanine or tyrosine and the indole glucosinolates synthesized from tryptophan (Grubb and Abel, 2006). Therefore, we evaluated the levels of these amino acids in both brassica species under Sb treatment conditions. Our results showed that Sb treatments significantly ($p<0.05$) enhanced the levels of amino acids such as methionine, leucine, tryptophan, and phenylalanine in both brassica species compared to the control, and the higher induction was recorded in Sb-treated *B. napus*. (Tab. 2). Methionine was the most dominant amino acid in both brassica species followed by phenylalanine, leucine, and tryptophan which were improved at *B. napus* by 1.52, 1.51, and 1.33-fold, and at *B. rapa* by (1.45, 1.72, 1.37 and 1.51-fold, respectively), higher than the value at the control (Tab. 2). It is well-recognized that the concentration of essential amino acids is one of the most critical variables determining plant nutritional value (Al Jaouni et al., 2018; Saleh et al., 2018). Methionine, for example, is an important nutrient in humans due to its function in choline extraction, lipid metabolism, and antioxidant defense system activation (Martinez et al., 2017). Furthermore, free amino acids serve as precursors to produce a variety of health-promoting compounds, including glucosinolates and phenolics. Where, phenylalanine is the substrate for the major enzyme in phenolic biosynthesis, phenylalanine ammonia-lyase (PAL) via the phenylpropanoid path. As a result, the availability of their amino acid precursor influences the production of bioactive metabolites such as glucosinolate (Grubb and Abel, 2006).

Glucosinolate is anticipated to be the most important bioactive substance in Brassicaceae species (Alotaibi et al., 2021). Increasing the levels of amino acid availability in Sb treated-Brassica species can explain the increased levels of glucosinolates compared to the corresponding controls (Tab. 2). Under v treatment, total glucosinolates were significantly ($p<0.05$) increased in *Brassica napus* and *B. rapa* compared to the control. Sb treated- *B. napus* showed the highest levels of total glucosinolate, glucoraphanin, progoitrin and sulforaphane which represented 1.52, 2.1, 2 and 1.87-fold, respectively, relative to their corresponding controls (Tab. 2). Furthermore, Sb treated-*B. rapa* showed higher content of the total glucosinolates, glucoraphanin, progoitrin and sulforaphane by 1.74, 1.6, and 1.37-fold, respectively.

Progoitrin have been identified as the one of the dominant aliphatic glucosinolate in both *B. napus and B. rapa*. Progoitrin is a precursor to goitrin, a compound known to have anti-thyroid properties, which could impact thyroid hormone metabolism in certain conditions (Chandra, 2020).

Under normal growth conditions, Brassica plants are also well-known for their high concentrations of isothiocyanates (ITCs) products, particularly sulforaphane (Almuhayawi et al., 2020). Where the biosynthesis of isothiocyanates (ITCs) products which included effective GLs (sulforaphane) and ineffective GLs (sulforaphane nitrile) is regulated by myrosinase enzyme through the hydrolysis process of glucosinolate (Williams et al., 2008). Herein, the level of sulforaphane was significantly ($p<0.05$) increased in Sb-treated *B. napus* and *B. rapa* by 62 and 63% of the values of corresponding controls, respectively (Tab. 2). Therefore, decreasing the ineffective glucosinolates hydrolysis products (sulforaphane nitrile) and increasing effective GLs (sulforaphane) under Sb treatment in both brassica species was explaining the nutritive impact of low Sb treatment in increasing the sulforaphane which considers a key factor in biological activities and nutritional values of Brassicaceae species. Accordingly, the synthesis of sulforaphane nitrile is promoted by epithiospecifier protein (ESP) which is generated from endogenous glucoraphanin instead of sulforaphane (Williams et al., 2008). In this context, Almuhayawi et al. (2020) showed that the synthesis of the health-promoting phytochemical sulforaphane is adversely linked with ESP activity in broccoli. The authors attributed that, the ESP enzyme is thought to be a critical role in the synthesis of sulforaphane nitrile in broccoli, therefore a cultivar with a low ESP expres-

Tab. 2: The overall amino acid levels and glucosinolates in control and Sb (8 mg/kg) treated *Brassica napus* and *Brassica rapa*. Data are represented by the means of five biological replicates ± standard error.

Amino Acids	*Brassica napus*		*Brassica rapa*	
	Control	Sb 8 mg/kg soil	Control	Sb 8 mg/kg soil
Amino acids (µmol /gDW)				
Alanine	1.19±0a^{b}	1.42±0.02^{a}	0.96±0.09^{b}	1.48±0.06^{a}
Leucine	8.3±0.24^{b}	11.1±0.06^{a}	7.03±0.24^{c}	9.7±0.09^{b}
Isoleucine	5.53±0.02^{b}	6.42±0.15^{a}	3.31±0.22^{c}	5.27±0.2^{b}
Valine	6.35±0.17^{a}	4.66±0.01^{b}	5.39±0.26^{ab}	6.01±0.42^{a}
Methionine	16.97±0.31^{c}	25.9±0.94^{a}	14.6±0.31^{c}	21.3±0.28^{ab}
Phenylalanine	9.15±0.23^{b}	13.9±1.03^{a}	7.19±0.05^{c}	12.4±0.43^{a}
Tyrosine	3.84±0.14^{ab}	4.75±0.16^{a}	3.29±0.05^{ab}	4.27±0.17^{a}
Tryptophan	5.72±0.08^{b}	9.85±0.11^{a}	3.31±0.24^{c}	5.02±0.22^{b}
Glucosinolates				
Total glucosinolates (µmol /gDW)	12.5±0.34^{b}	19.08±0.13^{a}	9.09±0.39^{c}	15.9±0.29^{b}
Progoitrin (µmol /gDW)	1.5±0.4^{a}	2.1 ±0.5^{d}	1.87±0.6^{b}	2.6±0.3^{c}
Glucoraphanin (nmol /gDW)	3.8±0.16^{c}	8.03±0.14^{a}	4.06±0.25^{c}	6.57±0.13^{b}
Sulforaphane nitrile (µmol /gDW)	3±0.06^{a}	1.88±0.13^{b}	2.58±0.3^{a}	1.63±0.05^{b}
Sulforaphane (µmol /gDW)	1.67±0.07^{b}	3.13±0.17^{a}	1.91±0.01^{b}	2.62±0.22^{a}

At $p<0.05$, various small letters (a, b, c...) within a row indicate significant differences between means.

sion level gathered more sulforaphane and showed higher potential as an anti-carcinogenic diet. Accordingly, the anticarcinogenic and anti-inflammatory effects of sprout extracts were enhanced by high glucoraphanin and sulforaphane levels in elevated CO_2-treated broccoli sprouts (Almuhayawi et al., 2020). Moreover, increasing the levels of total glucosinolates enhanced the anti-inflammatory, hypocholesterolemic, antibacterial, and anticancer properties of *Lepidium sativum* cultivars (Haraz, Khider, and Rajab) grown under elevated CO_2, and the activities were significantly different among the cultivars (Alotaibi et al. 2021).

Generally, Sb treatments increased the levels of amino acids in both treated *B. napus* and *B. rapa* resulting in increasing the total glucosinolates and dominant GLs glucoraphanin. Consequently, the treatment increased the value of effective GLs (sulforaphane) and decreased the ineffective GLs (sulforaphane nitrile), which could positively boost the biological activity and nutritional values of both brassica species under the low concentration of Sb treatments compared to the control.

Treatment of Sb increased the biological activities of *Brassica* species

The biological activities of plants are strongly linked to their endogenous bioactive phytochemicals. As a result of its positive effect on the accumulation of enzymatic antioxidants, carotenoids, total glucosinolates, and antioxidant metabolites, Sb treatment is predicted to influence the bioactivity of both v Treated- *B. napus* and *B. rapa*. Herein, *B. napus* and *B. rapa* showed similar increases in the levels of photosynthetic pigments, antioxidants, amino acids, and sulforaphane under the Sb treatment. Similarly, the antibacterial activities of Sb treated- *B. napus* and treated- *B. rapa* were significantly increased ($p<0.05$) compared to the control (Tab. 3). The antibacterial activities against *Streptococcus sp*., *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* were increased by 1.33, 1.46, 1.36, and 1.13-fold, respectively, compared to their respective controls. High antibacterial activities against *Escherichia coli*, and *Bacillus subtilis* (1.51 and 1.16-fold, respectively) were also observed (Tab. 3). Increasing the antibacterial activities of plant extracts are linked to their contents of bioactive isoflavonoids, phenolic acids, tocopherols, ASC, GSH, essential amino acids, and glucosinolate. For instance, the plant-made carotenoid pigments (β-carotene and β-cryptoxanthin) demonstrated antibacterial properties against *Vibrio parahaemolyticus*, *Escherichia coli*, *Bacillus subtilis*, and *Micrococcusluteus* (Boo et al., 2012). In this regard, carotenoids including carotene, lutein, and xanthophylls are well known for being potent ROS scavengers that increase their capability as antibacterial against several types of bacteria (Saleh et al., 2018). Overall, increasing the amounts of these pigments, amino acids, antioxidants

Tab. 3: The antibacterial activities of control and Sb (8 mg/kg) soil-treated *Brassica napus* and *Brassica rapa*. Data are represented by the means of five biological replicates ± standard error.

Anti-bacterial activity (zone inhibition (mm))	*Brassica napus*		*Brassica rapa*	
	Control	Sb 8 mg/kg soil	Control	Sb 8 mg/kg soil
Anti-bacterial (*Streptococcus* sp.)	17.16±0.96^{b}	22.95±1.06^{b}	13.29±0.79^{a}	13.08±0.61^{a}
Anti-bacterial (*Escherichia coli*)	16.99±0.45^{b}	24.92±0.2^{a}	15.51±1.22^{b}	23.54±0.06^{a}
Anti-bacterial *(Bacillus subtilis*)	15.93±1.17^{b}	21.7±0.33^{a}	14.84±0.77^{b}	17.27±0.81^{ab}
Anti-bacterial (*Pseudomonas aeruginosa)*	19.27±0.97^{a}	21.88±0.01^{a}	18.5±0.65^{a}	17.62±0.55^{b}

At $p<0.05$, various small letter superscripts (a, b, c...) within a row show significant differences between the control and Antimony-8 mg/kg soil-treated samples.

enzymes, metabolites, and total glucosinolate in Sb treated-*B. napus* and *B. rapa* are improving their biological activities as antibacterial by promoting antioxidants against the harmful production of ROS, which finally increases the beneficial secondary active metabolites and nutritional values of both brassica species.

Conclusion

In the current study, the low concentration of Sb at 8 mg/kg soil efficiently enhanced the growth of both *B. napus* and *B. rapa*, besides that it increased the levels of photosynthetic pigments such as chlorophyll a and b and carotenoids. The low concentration of Sb also increased the levels of several antioxidants such as tocopherol, phenolics, GSH, CAT, SOD, POX, and GR enzymes activities. The increase in amino acid contents in treated brassica species improved the levels of total glucosinolate and sulforaphane in treated brassica species compared to the control. Resulting in increasing the antibacterial activities of treated Brassica species against several types of bacteria. Finally, Sb treatment at low concentration (8 mg/kg soil) improves the growth and nutritional values of Brassica species as a source of valuable bioactive compounds.

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Conflict of interests

No potential conflict of interest was reported by the authors

References

Abdelgawad, H., Zinta, G., Hamed, B.A., Selim, S., Beemster, G., Hozzein, W.N., Wadaan, M.A.M., Asard, H., Abuelsoud, W., 2020: Maize roots and shoots show distinct profiles of oxidative stress and antioxidant defense under heavy metal toxicity. Environ. Pollut. 258, 113705. DOI: 10.1016/j.envpol.2019.113705

AbdElgawad, H., De Vos, D., Zinta, G., Domagalska, M.A., Beemster, G.T., Asard, H., 2015: Grassland species differentially regulate proline concentrations under future climate conditions: an integrated biochemical and modeling approach. New Phytol. 208 (2), 354-369. DOI: 10.1111/nph.13481

Al Jaouni, S., Saleh, A.M., Wadaan, M.A.M., Hozzein, W.N., Selim, S., AbdElgawad, H., 2018: Elevated CO_2 induces a global metabolic change in basil (*Ocimum basilicum* L.) and peppermint (*Mentha piperita* L.) and improves their biological activity. J. Plant Physiol. 224-225, 121-131. DOI: 10.1016/j.jplph.2018.03.016

Almuhayawi, M.S., Hassan, A.H.A., Al Jaouni, S.K., Alkhalifah, D.H.M., Hozzein, W.N., Selim, S., AbdElgawad, H., Khamis, G., 2021: Influence of elevated CO_2 on nutritive value and health-promoting prospective of three genotypes of Alfalfa sprouts (*Medicago Sativa*). Food Chem. 340, 128147.

Almuhayawi, M.S., AbdElgawad, H., Al Jaouni, S.K., Selim, S., Hassan, A.H.A, Khamis, G., 2020: Elevated CO_2 improves glucosinolate metabolism and stimulates anticancer and anti-inflammatory properties of broccoli sprouts. Food Chem. 328, 127102. DOI: 10.1016/j.foodchem.2020.127102

Alotaibi, M.O., Khamis, G., AbdElgawad, H., Mohammed, A.E., Sheteiwy, M.S., Elobeid, M.M., Saleh, A.M., 2021: *Lepidium sativum* Sprouts Grown under Elevated CO_2 Hyperaccumulate Glucosinolate and Antioxidants and Exhibit Enhanced Biological and Reduced Antinutritional Properties. Biomolecules. 11, 1174. DOI: 10.3390/biom11081174

Benzie, I.F.F., Strain, J.J., 1999: Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth. Enzymol. 15-27. DOI: 10.1016/s0076-6879(99)99005-5

Björkman, M., Klingen, I., Birch, A.N.E., Bones, A.M., Bruce, T.J.A., Johansen, T.J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L.E., 2011: Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. Phytochem. 72, 538-556. DOI: 10.1016/j.phytochem.2011.01.014

Boo, H.O., Hwang, S.J., Bae, C.S., Park, S.H., Heo, B.G., Gorinstein, S., 2012: Extraction and characterization of some natural plant pigments. Ind. Crops. Prod. 40 (1), 129-135. DOI: 10.1016/j.indcrop.2012.02.042

Chandra, A.K., 2010: Goitrogen in food: cyanogenic and flavonoids containing plant foods in the development of goiter. In: Bioactive foods in promoting health, 691-716. Academic Press.

Cuypers, A., Hendrix, S., Amaral, dos Reis, R., De Smet, S., Deckers, J., Gielen, H., Jozefczak, M., Loix, C., Vercampt, H., Vangronsveld, J., Keunen, E., 2016: Hydrogen peroxide, signaling in disguise during metal phytotoxicity. Front. Plant Sci. 7. DOI: 10.3389/fpls.2016.00470

DalCorso, G., Manara, A., Furini, A., 2013: An overview of heavy metal challenge in plants: from roots to shoots. Metall. 5, 1117e1132. DOI: 10.1039/c3mt00038a

Drotar, A., Fall, R., 1985: Methylation of xenobiotic thiols by *Euglena gracilis*: characterization of a cytoplasmic thiol methyltransferase. Plant and Cell Physiol. 26 (5), 847-854. DOI: 10.1093/oxfordjournals.pcp.a076979

Dhindsa, R.S., Plumb-Dhindsa, P.L., Reid, D.M., 1982: Leaf senescence and lipid peroxidation: effects of some phytohormones, and scavengers of free radicals and singlet oxygen. Physiol. Plant. 56 (4), 453-457. DOI: 10.1111/j.1399-3054.1982.tb04539.x

Duan, R., Lin, Y., Yang, L., Zhang, Y., Hu, W., Du, Y., Huang, M., 2023: Effects of antimony stress on growth, structure, enzyme activity and metabolism of Nipponbare rice (*Oryza sativa* L.) roots. Ecotoxicol. Environ. Saf. 249, 114409. DOI: 10.1016/j.ecoenv.2022.114409

Filella, M., Belzile, N., Chen, Y.W., 2001: Antimony in the environment: a review focused on natural water. I. Occurrence. Earth. Sci. Rev. 57, 125. DOI: 10.1016/S0012-8252(01)00070-8

Grubb, C.D., Abel, S., 2006: Glucosinolate metabolism and its control. Trends Plant Sci. 11, 89-100. DOI: 10.1016/j.tplants.2005.12.006

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999: Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207, 604-611. DOI: 10.1007/s004250050524

Jiang, Z.Y., Woollard, A.C.S., Wolff, S.P., 1990: Hydrogen peroxide production during experimental protein glycation. FEBS Lett. 268 (1), 69-71. DOI: 10.1016/0014-5793(90)80974-n

Khamis, G., Hamada, A.E., Schaarschmidt, F., Beemster, G.T.S., Asard, H., Papenbrock, J., 2019: Morphological and biochemical responses of *Balanites aegyptiaca* to drought stress and recovery are provenance-dependent. J. Agron. Crop. Sci. 205(5), 490-507. DOI: 10.1111/jac.12340

Kumar, K.B., Khan, P.A., 1982: Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv PR 202) leaves during senescence. Indian. J. Exp. Biol. 20 (5), 412.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994: Carbonyl assays for determination of oxidatively modified proteins. Meth. Enzymol. 233, 346-357. DOI: 10.1016/s0076-6879(94)33040-9

Liang, S.X., Gao, N., Li, X., Xi, X., 2018: Toxic effects of antimony on the seed germination and seedlings accumulation in *Raphanus sativus* L. radish and *Brassica napus*. Mol. Biol. Rep. 45, 2609-2614. DOI: 10.1007/s11033-018-4430-2

Liu, J., Zhang, J., Kim, S.H., Lee, H.S., Marinoia, E., Song, W.Y., 2021: Characterization of *Brassica rapa* metallothionein and phytochelatin synthase genes potentially involved in heavy metal detoxification. PLoS One. 16, 1-16. DOI: 10.1371/journal.pone.0252899

Martinez, Y., Li, X., Liu, G., Bin, P., Yan, W., Más, D., Valdivié, M., Hu, C.A.A., Ren, W., Yin, Y., 2017: The role of methionine on metabolism, oxidative stress, and diseases. Amino Acids 49, 2091-2098. DOI: 10.1007/s00726-017-2494-2

Miravet, R., Bonilla, E., Lopez-Sanchez, J.F., Rubio, R., 2005: Antimony speciation in terrestrial plants. Comparative studies on extraction methods. J. Environ. Monit. 7, 1207-1213. DOI: 10.1039/b509115b

Mostofa, M.G., Rahman, M.M., Siddiqui, M.N., Fujita, M., Tran, L.P., 2020: Salicylic acid antagonizes selenium phytotoxicity in rice: selenium homeostasis, oxidative stress metabolism and methylglyoxal detoxification. J. Hazard. Mater. 394, 122572. DOI: 10.1016/j.jhazm at.2020.122572

Rossi, G., Figliolia, A., Socciarelli, S., Pennelli, B., 2012: Capability of *Brassica napus* to accumulate cadmium, zinc and copper from soil. Acta Biotechnol. 22, 133-140. DOI: 10.1002/1521-3846(200205)22:1/2<133::AID-ABIO133>3.0.CO;2-3

Rout, G.R., Das, P., 2009: Effect of metal toxicity on plant growth and metabolism: I. Zinc. In: Lichtfouse, E., Navarrete, M., Debaeke, P., Veronique, S., Alberola, C. (eds.), Sustainable Agriculture, 873e884. Springer Netherlands, Dordrecht.

Posch, S., Bennett, L.T., 2009: Photosynthesis, photochemistry and antioxidative defence in response to two drought severities and with re-watering in *Allocasuarina luehmannii*. Plant Biol. 11, 83-93. DOI: 10.1111/j.1438-8677.2009.00245.x

Saleh, A.M., Selim, S., Al Jaouni, S., AbdElgawad, H., 2018: CO_2 enrichment can enhance the nutritional and health benefits of parsley (*Petroselinum crispum* L.) and dill (*Anethum graveolens* L.). Food Chem. 269, 519-526. DOI: 10.1016/j.foodchem.2018.07.046

Shtangeeva, I., Steinnes, E., Lierhagen, S., 2012: Uptake of different forms of antimony by wheat and rye seedlings. Environ. Sci. Pollut. Res. 19(2), 502-509. DOI: 10.1007/s11356-011-0589-y

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2004: Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant. Sci. 166, 293-302. DOI: 10.1016/j.plantsci.2003.09.018

Sønderby, I.E., Geu-Flores, F., Halkier, B.A., 2010: Biosynthesis of glucosinolate – gene discovery and beyond. Trends in Plant Sci. 15(5), 283-290. DOI: 10.1016/j.tplants.2010.02.005

Tschan, M., Robinson, B., Schulin, R., 2008: Antimony uptake by *Zea mays* (L.) and *Helianthus annuus* (L.) from nutrient solution. Environ. Geochem. Health. 30, 187-191. DOI: 10.1007/s10653-008-9142-4

Williams, D.J., Critchley, C., Pun, S., Nottingham, S., O'Hare, T.J., 2008: Epithiospecifier protein activity in broccoli: The link between terminal alkenyl glucosinolate and sulphoraphane nitrile. Phytochemistry. 69(16), 2765-2773. DOI: 10.1016/j.phytochem.2008.09.018

Zhou, X., Sun, C., Zhu, P., Liu, F., 2018: Effects of antimony stress on photosynthesis and growth of *Acorus calamus*. Front. Plant. Sci. 9, 579. DOI: 10.3389/fpls.2018.00579

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