Influence of soil properties on bioactive compounds and antioxidant capacity of *Brassica rupestris* Raf.

Running title: **Soil affects *Brassica* phytochemicals**.

Adele Muscolo[a]*, Maria Sidari[a], Giovanna Settineri[a], Teresa Papalia[a], Carmelo Mallamaci[a] Emilio Attinà[a]

[a] Dipartimento di Agraria, Università Mediterranea, Feo di Vito, 89124 Reggio Calabria, Italy

*Corresponding author:

Prof. Adele Muscolo

Agriculture Department

"Mediterranea" University of Reggio Calabria

Feo di Vito 89122, Reggio Calabria ITALY

Tel: 0039 09651694364

Fax: 003909651694550

e-mail: amuscolo@unirc.it

Orcid: 0000-0002-0439-1614

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30 **Abstract**

31 Purpose: *Brassica rupestri*s Raf., is a species native to South Italy, extremely rich in vitamins,
32 fibers and bioactive compounds with phytoterapic properties and may represent a resource for
33 pharmaceutical and nutraceutical industries. Thus, our aim was to evaluate if and which soil
34 properties affected the accumulation of secondary metabolites in *B. rupestris*. Knowledge of
35 the causes of plant metabolism changes in a specific area may represent an important
36 economic opportunity.

37 Methods: We analyzed soils (physical and chemical parameters) in two different locality of *B.*
38 *rupestris* growth, and leaves (biocompounds and antioxidant activity) of *B. rupestris* grown
39 over them to identify the soil factors that drove their phytochemical production and
40 antioxidant power.

41 Results: Soil properties influenced the contents but not the types of the bioactive compounds
42 in *B. rupestris.* No significant correlation was found between soil physical parameters and
43 phytochemicals in Brassica leaves. Conversely, soil chemical and biochemical properties
44 influenced the total antioxidant capacity and the synthesis of carotenoids and glucosinolates.
45 Soil organic matter (SOM), dehydrogenase activity (DH), fluorescein diacetate hydrolysis
46 (FDA), microbial biomass carbon (MBC) and humic acid/fulvic acid ratio (HA/FA) were the
47 most important soil factors influencing the amount of phytochemicals in *B. rupestris.*

48 Conclusions: A strict relationships between soil properties and metabolic profile of *B.*
49 *rupestris* was found. The synthesis of specific classes of metabolites in B. *rupestris* is a direct
50 response to soil biochemical conditions.

51 **Keywords:** Antioxidants; Biochemical indicators; *Brassica rupestris* Raf; Carotenoids; Soil
52 properties

1 Introduction

Secondary metabolites (SMs), synthesized by plants for adaptation to the environment and to cope with environmental stresses are an unique source of important bioactive compounds used in pharmaceutical, medical, nutritional and cosmetic fields (Siegler 1998) and more recently in nutraceutical industry (Kurlich et al. 1999; Podsedek 2007). Plant SMs on the basis of their chemical structures (Harborne 1999) are divided in several groups of large molecules, such as phenolic acids, flavonoids, terpenoids and steroids. The amount of these plant secondary products strongly depends on the environmental conditions which in turn affect the metabolic pathways responsible for the accumulation of the related natural products (Chrysargyris et al. 2017; Yang et al. 2018). Among the environmental conditions, soil in itself represents a complicated physical, chemical, and biological system, that not only determine whether a species will thrive, affecting its natural distribution, but also strongly influence growth, development and quality of plants (Liu et al. 2007; Yang et al. 2018; Zhang et al. 2019). Specifically, soil characteristics play a key role in the plant's ability to produce primary and secondary metabolites, and as reported by Ramakrishna and Ravishankar (2011) diverse soil conditions can cause significant differences in primary and secondary metabolite synthesis and accumulation in plants of the same species. Previous work evidenced a specificity between soil chemical characteristics and plant species (Muscolo et al. 2014) but also a direct effects of cultivation conditions on plant secondary metabolites (Boestfleisch et al. 2014; Panuccio et al. 2018). Brassicaceae vegetables are used as food in many areas worldwide, and are not only known for their high fiber, vitamin and mineral contents but also for their nutritional and pharmaceutical properties (Campbell et al. 2012). Some studies have recently demonstrated a relationship between a high consumption of *Brassica* and a reduced risk of cancer (Björkman et al. 2011; Wu et al. 2013), cardiovascular disease and diabetes (Kataya and Hamza 2008). *B. rupestris* is a species native to Calabria and Sicily, South Italy,

wild relative of *B*. *oleracea* vegetables, carrying the same C genome (n= 9) of the cultivated crops (Scialabba et al. 2010). It is extremely rich in a variety of secondary metabolites with nutraceutical and phytoterapic properties and as other brassicaceae is easily adaptable to a wide range of soil types and to many different climatic conditions (Erdem et al. 2010). Considering the rising attention to pharmacologically active plant-derived natural products and based on the fact that soil is a complex system that can create variation in the plant metabolite levels, our aim was to evaluate if and which soil properties affected the accumulation of secondary metabolites in *B. rupestris* because to have knowledge of the causes of plant metabolism changes in a specific area may represent an important economic opportunity. On this purpose, we analyzed soils in two different locality of *B. rupestris* growth and leaves of *B. rupestris* grown over them to identify the soil characteristics that drove their phytochemical production. Specific aim was to correlate the content of the single compounds to specific soil properties, to identify each soil factor which modulated the synthesis of bioactive compounds in brassica.

2 Materials and methods

2.1 Site description and soil sampling

The soils were collected in May 2017 in Calabria, South Italy, in two different locality: Stilo ("A", 38° 28' 34'' N, 16° 28' 02'' E; 400 m above sea level) and Pazzano ("B" 38° 28' 02'' N, 16° 27' 55'' E; 450 m above sea level) under *B. rupestris*. The climate is Mediterranean, with dry hot summers and cold winters. The annual mean temperature is 15.6 °C, with a mean rainfall of 905 mm. The soils in this zone developed from calcareous-dolomitic rocks. Soil in "A" is a *Dystric Regosol* with loamy sand texture; soil in "B" is an *Eutric Cambisol* with sandy loam texture (IUSS Working Group WRB 2006).

In each different locality, we identified five sites and in each sites, 6 replicates of topsoil (0-20 cm) were collected at a distance of 25 cm from the selected plants. Soil samples were air-dried and sieved (<2mm) before physical and chemical analysis. Fresh soil was used enzymatic analyses.

2.2 Soil analysis

Hydrometer method was used to determine the particle size distribution of soils, after dispersion with sodium hexametaphosphate (Bouyoucos 1962). Soil pH was measured with a pH-meter in a 2.5:1 water/soil ratio or in a 2.5:1 1M KCl/soil ratio. Soil organic carbon (SOC) was estimated following the method of Walkley and Black (1934). Soil organic matter (SOM) was calculated by multiplying soil organic carbon by 1.72. The ratio of humic and fulvic acid was performed as reported in Ciavatta and Govi (1993). Total nitrogen (TN, %) was measured by using the method of Bremner (1965). The conductometer was used to measure soil electrical conductivity (EC) in a 1:2.5 soil /water suspension. After distilled water extraction (Kaminsky and Muller 1978), total water soluble phenols (WSP) were measured by using Folin–Ciocalteau reagent (Box 1983) and expressed as tannic acid equivalents (μg TAET g^{-1}) (Kuiters and Denneman, 1987). Fluorescein diacetate hydrolysis (FDA) was measured following the method of Adam and Duncan (2001). The activity of dehydrogenase (DH) was detected with the method of von Mersi and Schinner (1991). Microbial Biomass Carbon (MBC) has been detected by following the chloroform fumigation extraction method (Vance et al. 1987) on moist samples (equivalent to 20 g dry wt.).

2.3 Plant analysis

In each different locality, we identified five sites and in each sites, seven upper leaves (the most tender) per each plant of *B. rupestris* were collected (6 replicates) and used for the secondary metabolite analyses.

2.4 Determination of total antioxidant capacity

The total antioxidant capacity (TAC) was determined spectrophotometrically by using the method of Prieto et al. (1999). Fresh leaves were ground and homogenized with distilled water (1:4 w/v) and centrifuged at 14,000 g for 30 minutes. The extracts were mixed with 1 mL of reagent solution (0.6 M H_2SO_4, 28 mM Na-phosphate, 4 mM NH_4-molybdate mixture), incubated for 90 min at 95 °C, and then cooled to room temperature. The absorbance was read at 695 nm. The results were expressed as µg tocopherol mL^{-1}

2.5 Glucosinolate extraction and determination

Glucosinolate was determined by high-performance liquid chromatography (HPLC), after desulphation on column. 20 mg of finely-ground leaf samples were treated with 400 µL of methanol, 10 µL of 0.3 M lead acetate, 120 µL of water and 12 µL of glucotropaeolin as internal standard. Extracts were centrifuged and supernatants were loaded on top of Sephadex column with the addition of 10 µL of water and 10 µL of sulfatase (Sigma-Aldrich, St. Louis). The columns were covered with aluminum foil and let to stand overnight at room temperature. Desulfoglucosinolates were eluted twice with 60% (v/v) methanol (100 µl) and then with water (100 µl). The chromatographic analyses were carried out on a HPLC Thermo Scientific Dionex Ultimate 3000, equipped with a C18 Thermofisher column (5-µm particle size, 150 × 4.8 mm i.d.) with a flow rate of 0.8 mL min^{-1} and temperature of 40 °C, and a C18 guard column (Thermofisher). Compounds were separated and detected at 229 nm following the method of Wathelet et al. (1991). Were quantified: glucobrassicin (GB, µM g^{-1} FW), glucoraphanin (GR, µM g^{-1}FW) and total glucosinolates (TG, µM g^{-1} FW).

2.6 Determination of Chlorophyll a and b

Chlorophyll *a* (Chl *a*) and *b* (Chl *b*) were determined grounding 50 mg of leaf tissues in 25 mL of absolute ethanol. Samples were mixed overnight in the dark at room temperature; afterwards, the supernatants were used for absorbance reading at 663 nm and 645 nm (Arnon 1949). Chlorophyll *a* and *b* content was expressed as mg 100 g^{-1} fresh weight (FW).

2.7 Determination of total phenolic content

Total phenol (TP) content was determined by colorimetry, using the Folin–Ciocalteu reagent diluted with distilled water in the 1:10 ratio (Singleton and Rossi 1965). 0.50 mL of the aqueous extract of the leaves was reacted with 0.5 mL of Folin–Ciocalteu reagent for 5 min, and then 2.5 mL of sodium hydroxide (0.33 N) was added to the reaction mixture. After 10 min incubation at room temperature the OD at 760 nm was read. Tannic acid was used as a reference standard, and the results were expressed as μg of tannic acid equivalents g^{-1} FW.

2.8 Quantification of carotenoids

Carotenoids (CAR) were extracted grinding 50 mg of leaf samples in 25 mL cold acetone (Lichtenthaler 1987). The mixture was incubated overnight in the dark at room temperature, then was centrifuged for 5 min at 14,000 rpm and the absorbance of samples was measured at 537, 647 and 663 nm. Carotenoids content was expressed as mg 100 g^{-1} FW.

2.9 Quantification of anthocyanins

For quantification of anthocyanins (ANT), 10 mg of leaf samples were ground in 1 mL of cold methanol/HCl/water (90:1:1, vol:vol:vol). Extraction was carried out under stirring, overnight, in the dark, at room temperature. The OD of extracts was measured at 529 and 650 nm. Results were expressed as μg anthocyanin g^{-1} FW (Murray and Hackett 1991).

177 **2.10 Determination of reduced glutathione, ascorbic and dehydroascorbic acids, tartaric**
178 **acid ester and flavonols**
179 Reduced glutathione (GSH) was extracted by grounding the leaf tissue with cold trichloracetic
180 acid. After centrifugation, the supernatant was incubated with a reaction mixture consisting of
181 potassium sulfate buffer pH 7.4 and 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB). The solution
182 was read at 412 nm on the spectrophotometer (Jollow et al. 1974).
183 Ascorbic (ASC) and dehydroascorbic (DHS) acids were determined following the method
184 of Law et al. (1983). Leaves were homogenized in a mortar with 5% metaphosphoric acid.
185 After centrifugation at the supernatant was added a phosphate buffer at pH 7.4 containing 5
186 mM ethylenediaminetetraacetic acid (EDTA) and 10 mM dithiothreitol (DTT). After 40 min
187 incubation at 40 °C, the activity was measured spectrophotometrically at 525 nm. The content
188 of ascorbic and dehydroascorbic acid was expressed as μg g^{-1} FW.
189 Esters of tartaric acid (TAE) and flavonols (FLA) were determined using the method of
190 Romani et al. (1996). Leaves were extracted in methanol (1:4, w/v) and centrifuged for 15
191 minutes at 14,000 g at 4 ° C. The supernatant was added to a reaction mixture composed by
192 225 μL 10% ethanol, 250 μL 0.1% HCl in 95% ethanol, 1 mL 2% HCl and 25μL of extract.
193 The esters of tartaric acid were measured at 320 nm and the flavonols at 360 nm. The tartaric
194 acid ester concentration was expressed as μg of caffeic acid g^{-1}FW, while the flavonols
195 amount as mg of quercetin g^{-1}FW.
196
197 **2.11 Statistical analysis**
198 Shapiro-Wilk's test was performed to test the assumption of normality. One-way analysis of
199 variance (ANOVA) was used to analyze all data, followed by Tukey's test at a 95%
200 confidence level. The Pearson's correlation coefficients were used to evaluate the correlation

between the active ingredients and the soil parameters studied. All statistical analyses were performed with SPSS 9.0 (SPSS, Chicago, IL, USA).

3 Results

3.1 Physicochemical soil properties

The soil properties changed between the two locality named "A" (Stilo) and "B" (Pazzano), under *B. rupestris* (Table 1). In all sites of the two locality, pH in KCl was significantly lower than that measured in H_2O. Soil pH was neutral in the sites of locality "B" (6.7), and moderately alkaline in the site of "A" (7.9). Both soils were not saline as evidenced by the low values of electrical conductivity (data not shown). The soils collected in "A" had lower content of organic matter (1.8%) and total nitrogen (0.18%) than soils collected in "B" (5.0% and 0.32%, respectively). The highest value of C/N ratio (9.1) was observed in "B". The highest activities of DH (24.5 μg TFF $g^{-1}h^{-}1$) and FDA (89 μg fluorescein $g^{-1}h^{-1}$) and the greatest content of WSP (145.50 μg TAET g^{-1}) were detected in soils of locality "B". The greatest amount of MBC was found in soils of locality "B", as well as the highest HA/FA ratio. Correlation analysis showed that SOM was linearly and positively correlated to DH, FDA, MBC and HA/FA. MBC was highly correlated to soil enzymes (DH and FDA), and HA/FA ratio was linearly correlated to SOM, DH, FDA and MBC.

3.2 Plant analysis

Leaf biomass and plant height (data not shown) were similar for the *B. rupestris* collected in both locality (Figure 1). The plants measured, in mean, 40 cm. Significant ($P \leq 0.05$) differences were instead observed in the antioxidant capacity and in the amount of chlorophylls between the plants collected in "A" and "B". The amount of total chlorophyll and chlorophyll a was the greatest in leaves of the plants grown in "B" (Table 2). Chlorophyll a/b ratio was lower in leaves of *B. rupestris* collected in "A" than in those collected in "B", as

well as lower was also the content of glucosinolates, carotenoids and anthocyanins. Conversely, *B. rupestris* grown in "A" contained the greatest amount of total phenols (Table 2) and tartaric acid esters (Table 3). No significant differences were observed in the flavonol content between the *B. rupestris* grown in "A" and "B". GSH, ASC, and TAC were significantly *(P*≤0.05) higher in leaves of *B. rupestris* grown in "B" than in "A". In particular, the TAC was 5.3 times higher in *B. rupestris* grown in "B" than in "A" (Table 3). No linear correlation among plant bioactive compounds has been found, instead a positive and significant correlation (P<0.01) between soil parameters (SOM, DH, FDA, MBC and HA/FA) and plant bioactive compounds (GSH, TAC, ASC, CAR and GR, GB and TG) was really evident (Table 4). No significant correlation was found instead between the soil physical parameters and the active compounds in *Brassica* leaves (data not shown).

4 Discussion

Generally, plants respond to changes in the ecosystem by altering their morphology, physiology, and by producing secondary metabolites implicated in plant defense from (Heath and Boller 2002; Gratani 2014) the overcoming stress conditions (Bourgaud et al. 2001). In previous works an increase in valuable plant secondary metabolites as consequence of alteration in the growing media in seedlings and plants from different families (*Amaranthaceae, Brassicaceae, Plantaginaceae and Rhizophoraceae*) and habitats was observed (Boestfleisch et al 2014). This could be considered as a good opportunity to increase their nutritional and economic values. Hepperly et al. (2018) showed a positive correlation between soil fertility, plant adaptability, nutrients and antioxidants. In the recent years, there was a great interest in plant secondary metabolites for their medicinal and aromatic properties (Marchese and Rehder 2001; Ramakrishna and Ravishankar 2011). The highlighted different biochemical response of *B. rupestris* to the two soils with diverse characteristics, was in

agreement with previous findings of James et al. (2005) and Omeňo and Fernandez (2012), showing that soil, with its intrinsic characteristics, is directly responsible for plant metabolite production. A part soil organic matter and pH that are well known to be indicators of fertility driving the nutrient availability (Läuchli and Grattan 2012; Nwite 2017) we analyzed the labile fraction of organic matter in particular WSP, MBC, FDA, DH and HA/FA ratio that are important biological parameters of soil ecosystem functioning and soil quality (Muscolo et al. 2014). Microbial biomass is considered indicator of fertility because it is involved in C utilization; FDA, hydrolyzed by the enzymes involved in microbial activity (e.g., esterases, proteases, and lipases), is strictly correlated with microbial biomass and thus it is used as representative of hydrolytic soil activity (Adam and Duncan 2001). DHs are the major intracellular enzymes representative of the oxidoreductase class (Gu et al. 2009) and are used as marker of microbial oxidoreduction processes (Moeskops et al. 2010). WSP and HA/FA ratio are related to the dynamic of organic matter in soil (Min et al. 2015). Our data evidenced that soils scarce in organic matter and nitrogen ("A"), with low microbial biomass and HA/FA ratio, induced physio-metabolic stress in plants causing a marked reduction in photosynthetic performance by altering the ultrastructure of the organelles and the concentration of various photosynthetic pigments as already previously shown by Asharaf and Harris (2013). The decrease in the synthesis of chlorophylls, carotenoids and anthocyanins in *B. rupestris* grown on soil with scarce fertility ("A") showed that soil fertility directly influenced pigment production in plants, confirming previous findings of Pal et al. (2015) that showed as soil fertility affected secondary metabolite production in *Stevia rebaudiana.* The low amount of MBC and enzyme activities found in "A" contributed to a reduced degradation of SOM with consequent scarce availability of nutrients, particularly nitrogen, that has an important role in the synthesis of chlorophylls and in the assimilative process as already demonstrated by other authors (Grassmann et al. 2002; Mittler 2002; Blokhina et al. 2003).

Carotenoids and their oxidative products (apocarotenoids), with a key role in various biological processes such as assembly of photosystems, photo protection and regulation of growth and development (Cazzonelli and Pogson 2010; Havaux 2014), were also strongly affected by the quantity of SOM. Carotenoids, implicated in the interactions of plants with their environment (Cazzonelli 2011; Walter and Strack 2011) considered also marker of stress conditions, were in fact lower in the *B. rupestris* grown on the less fertile soils. The lowest amount of leaf anthocyanins in *B. rupestris* grown in "A" confirmed previous findings of Kovinich et al. (2015) showing that anthocyanins were induced in plant vegetative tissues in response to a number of different abiotic stresses such as drought, salinity, excess of light, sub- or supra-optimal temperatures, and nitrogen and phosphorous deficiency. Nitrogen is an essential nutrient and plants can survive to a limiting nitrogen (N) supply only by developing a series of adaptive responses (Peng et al. 2008). As demonstrated by Peng and coworkers (2008) *Arabidopsis* mutant grown with limiting N produced significantly lower anthocyanin amounts (particularly cyanidins) increasing, in parallel, lignin content. The increase in total phenols in leaves of *B. rupestris* grown in the less fertile soils, evidenced that the plants were under stress, and that the carbon fixed with photosynthesis, was predominantly allocated to produce secondary metabolites more than leaf pigments (Winkel-Shirley 2001). The glucosinolates were almost higher in *B. rupestris* grown in "B" than in "A", as result of the major content of soil organic matter. These results were in agreement with Naguib et al. (2012) that found a greater amount of total glucosinolates in broccoli amended with organic and bioorganic fertilizers. Our results showed clearly a positive and significant correlation between soil chemical and biological properties and plant pigment synthesis. The set of analytical techniques utilized, combined with proper statistical analysis, allowed us to evidence a strict relationships between soil properties and metabolic profile of *B. rupestris* and to highlight the role that soil biochemical characteristics play on the metabolite

301 production in Brassica, evidencing that soils with a very high content of organic matter (>4%)
302 and balanced oxidoreductasic and hydrolytic enzymatic activities can positively affect
303 brassica phytochemical synthesis with important impact in food and pharmaceutical industries
304 and positive repercussions on the local bio-economy sector.

305

306 ## 5 Conclusions

307 In short, the effects of compounds of vegetable origin, which in plants play important
308 ecological functions, useful for competition and survival of the species, are extremely
309 inhomogeneous both in chemical and functional terms, but they are united by having a low
310 molecular weight and cannot be synthesized by humankind on which they generally perform a
311 synergistic or complementary action with other substances. Minor plant species or species
312 and/or varieties that are no longer cultivated, because they are not appreciated by the market
313 or because they are not very productive, can represent a natural reserve of these bioactive
314 compounds and could be adequately valorized with significant commercial effects in the
315 functional food sector or in the pharmacological field.

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320

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Figure Caption

Figure 1. *Brassica rupestris* grown in site "A" (Stilo) and in site "B" (Pazzano).

Table 1 Soil physical, chemical and biochemical properties of two different localities ("A" and " B") under *Brassica rupestris*: texture; pH (H_2O); pH (KCl); soil organic matter (SOM, %); total nitrogen (TN, %); carbon/nitrogen ratio (C/N); dehydrogenase (DH, µg TFF $g^{-1}h^{-1}$); fluorescein diacetate (FDA, µg fluorescein $g^{-1}h^{-1}$); water soluble phenols (WSP, µg TAET g^{-1}), microbial biomass carbon (MBC, mg C g^{-1} f. s.), humic acid/fulvic acid ratio (HA/FA). Numbers in parentheses denote the SD of the mean (n = 30).

Site	**Depth (cm)**	**Texture**	**pH (H_2O)**	**pH (KCl)**	**SOM**	**TN**	**C/N**	**DH**	**FDA**	**WSP**	**MBC**	**HA/FA**
"A"	0-20	Loamy sand	7.9(0.03)a*	7.1(0.02)a	1.8(0.2)b	0.18(0.02)b	5.9(0.1)b	12.4(0.8)b	70(5)b	112.51(7)b	889(13)b	0.80(0.05)b
"B"	0-20	Sandy loam	6.7(0.04)b	5.8(0.04)b	5.0(0.5)a	0.32(0.01)a	9.1(0.4)a	24.5(0.6)a	89(3)a	145.50(7)a	1010(11)a	1.84(0.02)a

*Different letters in the same column indicate significant differences $P \leq 0.05$.

Table 2 Content of anthocyanins (ANT, µg anthocyanin g^{-1}FW), chlorophyll *a* (Chl *a,* mg 100 g^{-1}FW), chlorophyll *b* (Chl *b,* mg 100 g^{-1}FW), total chlorophyll (TChl, mg 100 g^{-1}FW), chlorophyll *a/* chlorophyll *b* (Chl*a*/Chl*b*), carotenoids (CAR, mg 100 g^{-1}FW) and total phenols (TP, µg of tannic acid g^{-1} FW) in leaves of *Brassica rupestris.* Numbers in parentheses denote the SD of the mean (n = 30).

Site	**ANT**	**Chl *a***	**Chl *b***	**TChl**	**Chl*a*/Chl*b***	**CAR**	**TP**
"A"	2.4(0.3)b*	94.7(3)b	60.6(0.8)a	155.3(0.9)b	1.56(0.2)b	8.0(0.7)b	4342(20)a
"B"	5.9(0.1)a	112.7(2)a	55.2(0.9)b	167.9(0.9)a	2.04(0.1)a	16.1(0.3)a	3113(16)b

*Different letters in the same column indicate significant differences P≤0.05.

503 **Table 3** Content of tartaric acid ester (TAE, µg caffeic acid g^{-1} FW), flavonoids (FLA, mg
504 quercetin g^{-1} FW), reduced glutathione (GSH, µg g^{-1} FW), ascorbic acid (ASC, µg g^{-1} FW),
505 dehydro ascorbic acid (DHS, µg g^{-1}FW), glucobrassicin (GB, µM g^{-1} FW) , glucoraphanin
506 (GR, µM g^{-1}FW), total glucosinolates (TG, µM g^{-1} FW) and total antioxidant activity (TAC,
507 µg tocopherol mL^{-1}) in leaves of *Brassica rupestris.* Numbers in parentheses denote the SD of
508 the mean (n = 30).

Phytochemicals	**Site "A"**	**Site "B"**
TAE	12.3(0.1)a*	10.6(0.3)b
FLA	0.00999(0.0001)a	0.01003(0.0001)a
GSH	150.9(2)b	187.0(1)a
ASC	65.8(0.7)b	150.2(2)a
DHS	92.6(2)a	96.1(1)a
GB	7(0.3)b	16(0.5)a
GR	2.8(0.07)b	5.7(0.1)a
TG	11(0.8)b	16(0.5)[a]
TAC	462.0(11)b	2442(27)a

509 * Different letters in the same row indicate significant differences at P≤0.05.

Table 4 Matrix correlation between active ingredients and soil parameters. Soil organic matter (SOM), total nitrogen (TN), dehydrogenase (DH), fluorescein diacetate (FDA), water soluble phenols (WSP), microbial biomass carbon (MBC), humic acid/fulvic acid ratio (HA/FA), ascorbic acid (ASC), total antioxidant activity (TAC), reduced glutathione (GSH), tartaric acid ester (TAE), total chlorophyll (TChl), carotenoids (CAR), glucoraphanin (GR), glucobrassicin (GB), total glucosinolates (TG).

	SOM	TN	DH	FDA	WSP	MBC	HA/FA	ASC	TAC	GSH	TAE	TChl	CAR	GR	GB	TG
SOM	1															
TN	0.68	1														
DH	0.86**	0.44	1													
FDA	0.99**	0.41	0.71	1												
WSP	0.66	0.47	0.58	0.66	1											
MBC	0.97**	0.40	0.87**	0.90**	0.52	1										
HA/FA	0.97**	0.43	0.90**	0.90**	0.57	0.97**	1									
ASC	0.89**	0.57	0.86**	0.95**	0.44	099**	0.87**	1								
TAC	0.98**	0.68	0.98**	0.98**	0.41	0.90**	0.92**	0.37	1							
GSH	0.98**	0.57	0.99**	0.65	0.37	0.99**	0.87**	0.45	0.27	1						
TAE	-0.68	-0.39	-0.69	-0.70	-0.49	-0.45	-0.48	-0.44	-0.41	-0.33	1					
TChl	0.65	0.47	0.99**	0.69	0.39	0.90**	0.87**	0.54	0.47	0.63	-0.56	1				
CAR	0.98**	0.55	0.99**	0.92**	0.17	0.97**	0.86**	0.68	0.41	0.48	-0.28	0.37	1			
GR	0.97**	0.34	0.99**	0.87*	0.67	0.96**	0.90**	0.33	0.55	0.61	-0.34	0.43	0.68	1		
GB	0.99**	0.56	0.95**	0.98**	0.51	0.99**	0.95**	0.28	0.27	0.51	-0.69	0.42	0.53	0.40	1	
TG	0.96**	0.44	0.98**	0.97**	0.37	0.98**	0.90**	0.23	0.41	0.39	-0.56	0.58	0.57	0.37	0.47	1

**P<0.01

Site “A”

Site “B”

Fig 1.