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Influence of Soil Properties on Bioactive Compounds and Antioxidant Capacity of Brassica rupestris Raf

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

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

4
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21 Author Contributions: AM conceived and designed the experiments, analyzed the data, wrote
22 the manuscript. MS analyzed the data, performed statistical analyses and critically reviewed
23 and edited the manuscript. GS and TP performed the experiments, collected and analyzed the
24 data. EA and CM conducted fieldwork.

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29

30 **Abstract**

31 Purpose: *Brassica rupestris* 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

53 **1 Introduction**

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

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

92

93 **2 Materials and methods**

94 **2.1 Site description and soil sampling**

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

102 In each different locality, we identified five sites and in each sites, 6 replicates of topsoil (0-
103 20 cm) were collected at a distance of 25 cm from the selected plants. Soil samples were air-
104 dried and sieved (<2mm) before physical and chemical analysis. Fresh soil was used
105 enzymatic analyses.

106 **2.2 Soil analysis**

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

122

123 **2.3 Plant analysis**

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

127

128 **2.4 Determination of total antioxidant capacity**

129 The total antioxidant capacity (TAC) was determined spectrophotometrically by using the
130 method of Prieto et al. (1999). Fresh leaves were ground and homogenized with distilled
131 water (1:4 w/v) and centrifuged at 14,000 g for 30 minutes. The extracts were mixed with 1
132 mL of reagent solution (0.6 M H₂SO₄, 28 mM Na-phosphate, 4 mM NH₄-molybdate mixture),
133 incubated for 90 min at 95 °C, and then cooled to room temperature. The absorbance was read
134 at 695 nm. The results were expressed as µg tocopherol mL⁻¹

135

136 **2.5 Glucosinolate extraction and determination**

137 Glucosinolate was determined by high-performance liquid chromatography (HPLC), after
138 desulphation on column. 20 mg of finely-ground leaf samples were treated with 400 µL of
139 methanol, 10 µL of 0.3 M lead acetate, 120 µL of water and 12 µL of glucotropaeolin as
140 internal standard. Extracts were centrifuged and supernatants were loaded on top of Sephadex
141 column with the addition of 10 µL of water and 10 µL of sulfatase (Sigma-Aldrich, St. Louis).
142 The columns were covered with aluminum foil and let to stand overnight at room
143 temperature. Desulfoglucosinolates were eluted twice with 60% (v/v) methanol (100 µl) and
144 then with water (100 µl). The chromatographic analyses were carried out on a HPLC Thermo
145 Scientific Dionex Ultimate 3000, equipped with a C18 Thermofisher column (5-µm particle
146 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
147 guard column (Thermofisher). Compounds were separated and detected at 229 nm following
148 the method of Wathelet et al. (1991). Were quantified: glucobrassicin (GB, µM g⁻¹ FW),
149 glucoraphanin (GR, µM g⁻¹FW) and total glucosinolates (TG, µM g⁻¹ FW).

150

151 **2.6 Determination of Chlorophyll a and b**

152 Chlorophyll *a* (Chl *a*) and *b* (Chl *b*) were determined grinding 50 mg of leaf tissues in 25
153 mL of absolute ethanol. Samples were mixed overnight in the dark at room temperature;
154 afterwards, the supernatants were used for absorbance reading at 663 nm and 645 nm (Arnon
155 1949). Chlorophyll *a* and *b* content was expressed as mg 100 g⁻¹ fresh weight (FW).

156

157 **2.7 Determination of total phenolic content**

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

164

165 **2.8 Quantification of carotenoids**

166 Carotenoids (CAR) were extracted grinding 50 mg of leaf samples in 25 mL cold acetone
167 (Lichtenthaler 1987). The mixture was incubated overnight in the dark at room temperature,
168 then was centrifuged for 5 min at 14,000 rpm and the absorbance of samples was measured at
169 537, 647 and 663 nm. Carotenoids content was expressed as mg 100 g⁻¹ FW.

170

171 **2.9 Quantification of anthocyanins**

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

176

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 trichloroacetic
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 $\mu\text{g g}^{-1}$ 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 25 μL 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

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

203

204 **3 Results**

205 **3.1 Physicochemical soil properties**

206 The soil properties changed between the two locality named “A” (Stilo) and “B” (Pazzano),
207 under *B. rupestris* (Table 1). In all sites of the two locality, pH in KCl was significantly
208 lower than that measured in H₂O. Soil pH was neutral in the sites of locality “B” (6.7), and
209 moderately alkaline in the site of “A” (7.9). Both soils were not saline as evidenced by the
210 low values of electrical conductivity (data not shown). The soils collected in “A” had lower
211 content of organic matter (1.8%) and total nitrogen (0.18%) than soils collected in “B” (5.0%
212 and 0.32%, respectively). The highest value of C/N ratio (9.1) was observed in “B”. The
213 highest activities of DH (24.5 $\mu\text{g TFF g}^{-1}\text{h}^{-1}$) and FDA (89 $\mu\text{g fluorescein g}^{-1}\text{h}^{-1}$) and the
214 greatest content of WSP (145.50 $\mu\text{g TAET g}^{-1}$) were detected in soils of locality “B”. The
215 greatest amount of MBC was found in soils of locality “B”, as well as the highest HA/FA
216 ratio. Correlation analysis showed that SOM was linearly and positively correlated to DH,
217 FDA, MBC and HA/FA. MBC was highly correlated to soil enzymes (DH and FDA), and
218 HA/FA ratio was linearly correlated to SOM, DH, FDA and MBC.

219 **3.2 Plant analysis**

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

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

237

238 **4 Discussion**

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

251 agreement with previous findings of James et al. (2005) and Omeño and Fernandez (2012),
252 showing that soil, with its intrinsic characteristics, is directly responsible for plant metabolite
253 production. A part soil organic matter and pH that are well known to be indicators of fertility
254 driving the nutrient availability (Läuchli and Grattan 2012; Nwite 2017) we analyzed the
255 labile fraction of organic matter in particular WSP, MBC, FDA, DH and HA/FA ratio that are
256 important biological parameters of soil ecosystem functioning and soil quality (Muscolo et al.
257 2014). Microbial biomass is considered indicator of fertility because it is involved in C
258 utilization; FDA, hydrolyzed by the enzymes involved in microbial activity (e.g., esterases,
259 proteases, and lipases), is strictly correlated with microbial biomass and thus it is used as
260 representative of hydrolytic soil activity (Adam and Duncan 2001). DHs are the major
261 intracellular enzymes representative of the oxidoreductase class (Gu et al. 2009) and are used
262 as marker of microbial oxidation processes (Moeskops et al. 2010). WSP and HA/FA
263 ratio are related to the dynamic of organic matter in soil (Min et al. 2015). Our data evidenced
264 that soils scarce in organic matter and nitrogen (“A”), with low microbial biomass and
265 HA/FA ratio, induced physio-metabolic stress in plants causing a marked reduction in
266 photosynthetic performance by altering the ultrastructure of the organelles and the
267 concentration of various photosynthetic pigments as already previously shown by Asharaf and
268 Harris (2013). The decrease in the synthesis of chlorophylls, carotenoids and anthocyanins in
269 *B. rupestris* grown on soil with scarce fertility (“A”) showed that soil fertility directly
270 influenced pigment production in plants, confirming previous findings of Pal et al. (2015) that
271 showed as soil fertility affected secondary metabolite production in *Stevia rebaudiana*. The
272 low amount of MBC and enzyme activities found in “A” contributed to a reduced degradation
273 of SOM with consequent scarce availability of nutrients, particularly nitrogen, that has an
274 important role in the synthesis of chlorophylls and in the assimilative process as already
275 demonstrated by other authors (Grassmann et al. 2002; Mittler 2002; Blokhina et al. 2003).

276 Carotenoids and their oxidative products (apocarotenoids), with a key role in various
277 biological processes such as assembly of photosystems, photo protection and regulation of
278 growth and development (Cazzonelli and Pogson 2010; Havaux 2014), were also strongly
279 affected by the quantity of SOM. Carotenoids, implicated in the interactions of plants with
280 their environment (Cazzonelli 2011; Walter and Strack 2011) considered also marker of stress
281 conditions, were in fact lower in the *B. rupestris* grown on the less fertile soils. The lowest
282 amount of leaf anthocyanins in *B. rupestris* grown in “A” confirmed previous findings of
283 Kovicich et al. (2015) showing that anthocyanins were induced in plant vegetative tissues in
284 response to a number of different abiotic stresses such as drought, salinity, excess of light,
285 sub- or supra-optimal temperatures, and nitrogen and phosphorous deficiency. Nitrogen is an
286 essential nutrient and plants can survive to a limiting nitrogen (N) supply only by developing
287 a series of adaptive responses (Peng et al. 2008). As demonstrated by Peng and coworkers
288 (2008) *Arabidopsis* mutant grown with limiting N produced significantly lower anthocyanin
289 amounts (particularly cyanidins) increasing, in parallel, lignin content. The increase in total
290 phenols in leaves of *B. rupestris* grown in the less fertile soils, evidenced that the plants were
291 under stress, and that the carbon fixed with photosynthesis, was predominantly allocated to
292 produce secondary metabolites more than leaf pigments (Winkel-Shirley 2001). The
293 glucosinolates were almost higher in *B. rupestris* grown in “B” than in “A”, as result of the
294 major content of soil organic matter. These results were in agreement with Naguib et al.
295 (2012) that found a greater amount of total glucosinolates in broccoli amended with organic
296 and bioorganic fertilizers. Our results showed clearly a positive and significant correlation
297 between soil chemical and biological properties and plant pigment synthesis. The set of
298 analytical techniques utilized, combined with proper statistical analysis, allowed us to
299 evidence a strict relationships between soil properties and metabolic profile of *B. rupestris*
300 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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481 Figure Caption

482

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

484 **Table 1** Soil physical, chemical and biochemical properties of two different localities (“A” and “ B”) under *Brassica rupestris*: texture; pH
 485 (H₂O); pH (KCl); soil organic matter (SOM, %); total nitrogen (TN, %); carbon/nitrogen ratio (C/N); dehydrogenase (DH, µg TFF g⁻¹h⁻¹);
 486 fluorescein diacetate (FDA, µg fluorescein g⁻¹h⁻¹); water soluble phenols (WSP, µg TAET g⁻¹), microbial biomass carbon (MBC, mg C g⁻¹ f. s.),
 487 humic acid/fulvic acid ratio (HA/FA). Numbers in parentheses denote the SD of the mean (n = 30).

Site	Depth (cm)	Texture	pH (H ₂ O)	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

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

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

495

496	Site	ANT	Chl <i>a</i>	Chl <i>b</i>	TChl	Chl <i>a</i> /Chl <i>b</i>	CAR	TP
498	“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
499	“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

500

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

502

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 $P \leq 0.05$.

510 **Table 4** Matrix correlation between active ingredients and soil parameters. Soil organic matter (SOM), total nitrogen (TN), dehydrogenase
 511 (DH), fluorescein diacetate (FDA), water soluble phenols (WSP), microbial biomass carbon (MBC), humic acid/fulvic acid ratio (HA/FA),
 512 ascorbic acid (ASC), total antioxidant activity (TAC), reduced glutathione (GSH), tartaric acid ester (TAE), total chlorophyll (TChl), carotenoids
 513 (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	0.99**	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

514 **P<0.01

515



Site "A"

516

517

518 Fig 1.



Site "B"