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Integrated Approach to the Auto-Ecological Study of Presence of *Brassica incana* Ten. in the Gargano Promontory (Puglia, Southern Italy), on the Adriatic Side of the Italian Peninsula

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I. Introduction

The research focuses on the analysis of Brassica *incana*, a species of the Apulian flora, whose presence (Conti, 2005; Bartolucci et al. 2018) is based on several reports for the Tremiti Islands (Béguinot 1906; De Marco et. 1984, Maggioni et al. 1996, 2008, Licht-2020) and another for Salento (Groves 1887), considered extinct in the latest update of "Flora del Salento" (Mele et al. 2006). For the Gargano it was not listed in the Prodromus Florae Garganicae by Fenaroli (1966-1974) but will then be described in a recent analytical flora (Licht 2008) documented by a herbarium sheet (MJG herbarium–Licht-2017) of samples collected from Peschici and San Menaio.

In light of these reports, certainly not exhaustive, and in consideration of a little known species in Italy, it was intended to deepen the knowledge of the populations present on the Gargano Promontory which, together with those of the Tremiti Islands, have phytogeographic value, being the only presences on the Adriatic side of the Italian peninsula.

Its distribution range in Italy is limited to the southern regions with its northern limit on the Adriatic side at the Gargano, it also occurs on the Tyrrhenian side in Tuscany (Bartolucci et al. 2018). The species also occurs along the coasts of the Balkan countries including Croatia (Maggioni et al. 1996; Snogerup et al. 1990), Slovenia, Bosnia Herzegovina, Serbia and Montenegro (Snogerup et al. 1990). Therefore, being a real amphi-Adriatic species, it is part of the conspicuous group of species that testifies the ancient paleogeological connections between Apulia and the Balkan peninsula. Furthermore, *B. incana* is part of a large group of eastern Mediterranean species that have their eastern limit in Albania (Proko 1988).

The interest of the research was to find a possible relationship with geological factors that explained the presence of the species so rare and localized on the Gargano.

a) Botany, taxonomy and ethnobotany of the species

Brassica incana is a suffruticose chamaephyte that reaches 150 cm in height with long and thick woody stems (up to 2 cm thick). The pubescent, whitish, poorly structured leaves have a soft and hairy texture due to the presence of hairs, generally simple and denser along the veins. The lower leaves (basal) have winged stalks (10 cm), smaller than the lamina that reaches remarkable size (8-15 x 12-25 cm), having a variable shape (lanceolate, lirate, auricolate or amplexicauli ovate) with margins irregularly denticulate; upper undivided leaves are gradually smaller. Flowers are grouped into racemes having a linear-lengthened development (10-30 cm) rich of many yellow flowers, bleaching in the dry (pedicels 10-25 mm; yellowish sepals 12-16 x 2-4 mm; yellow-bright petals 20-30 x 8-13 mm). The silique is arched (slightly compressed dorsally), more than 5 cm long; the seeds are 3-4 x 4-5 mm is conic 8-14 mm long, seedless rostrum. Flowering occurs in March-April. The chromosome number is 2n=18 (Ferrarella & al. 1980; Pignatti, 1982;

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2018). In the past, the species was described as a sub-variety [*B. oleracea* subvar. *incana* (Ten.) Cosson] (Tenore 1811-1815) or a variety of *Brassica oleracea* [*B. oleracea* var. *incana*(Ten.) Paol.] (Fiori 1896-1908), or even as a subspecies of *B. sylvestris* [*B. sylvestris* subsp. *incana* (Ten.) Onno] (Fiori 1923-1925). The recent taxonomy raises it to species value named *B. incana* (Pignatti 1982, 2018; Bartolucci et al. 2018). *B. incana* is an edible species; in Italy it is known by rural communities as cabbage or "wild" broccoli (Campania, Ischia, Calabria), but in the etno botanic literature there are no reports about uses as food, contrary to other species of the same genus, such as *Brassica nigra* (L.) W.D.J.Koch (Umbria, Apulia, Basilicata, Sicily and Sardinia), *Brassica rupestris* Raf. subsp. *rupestris* (Sicily), *Brassica tournefortii* Gouan (Sicily), *Brassica tyrrhena* Giotta, Piccitto & Arrigoni (Sardinia) (Biscotti et al. 2018). All the species of genus *Brassica*, especially *Brassica oleracea*, are considered to be possible ancestors of many cultivated vegetables (cabbages, broccoli, etc.) marketed all over the world; *B. incana* has also been studied as a potential relative of cultivated cabbages having probably crossbreed with *B. oleracea* (Dixon 2007; Hammer et al. 2013; Tsunoda et al. 1980; Gigante et al. 2012).

b) Italian distribution range

According to Flora d'Italia (Pignatti 1982), the species is recorded in Campania, Basilicata, Calabria, Apulia, Sicily (including the islands) and the Pontian Archipelago. Its occurrence were recently confirmed in Basilicata (Conti et al. 2004; Lucca et al. 2004), Calabria (Muscolo et al. 2017) and Sicily (Castellano et al. 2009). In the latter region, the species is present on the northern and eastern sectors of the island even if the old record of the species in Trapani was attributed to *Brassica villosa* subsp. *bivoniana*(Mazzola & Raimondo) Raimondo & Mazzola or *Brassica villosa* subsp. *drepanensis* (Caruel) Raimondo & Mazzola (Ottonello et al. 1958; Raimondo et al. 1991). Finally, several well-known occurrences in different Italian islands are known (Giglio, Ponza, Napoli archipelago, Aeolian archipelago, Tremiti, Pelagosa, and Pelagie Islands) (De Natale 2003). Therefore, the Italian distribution range of *B. incana* is disjointed and limited to some localities of the Tyrrhenian and Ionian sides such as Tuscany, Lazio, Campania, Basilicata, Calabria and Sicily; for the Adriatic side it is known only in Apulia (Conti et al. 2005; Bartolucci et al. 2018; Pignatti 2018). The Italian distribution is shown in Figure 1.

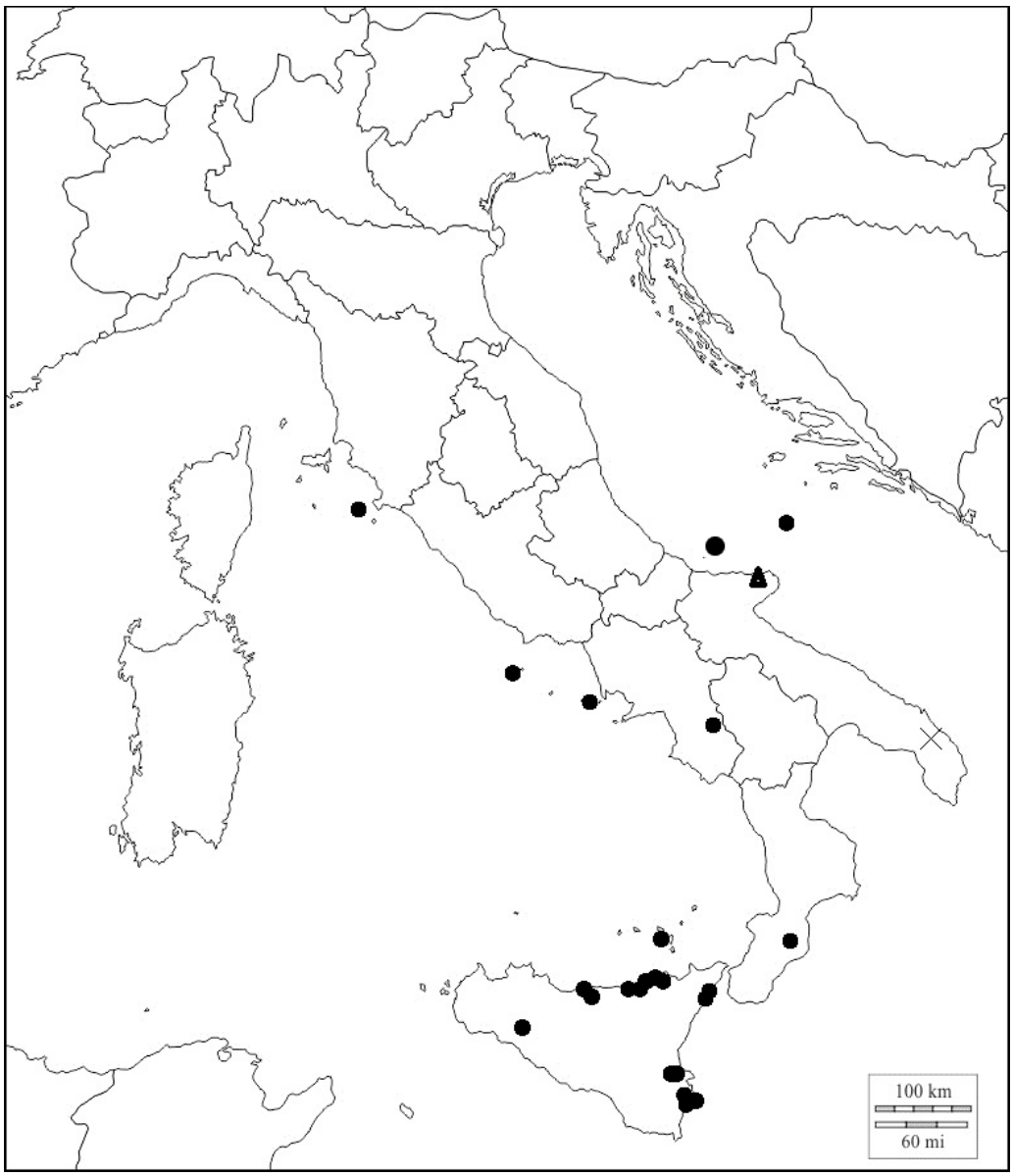

Figure 1: Distribution range of *B. incana* in Italy. The dot indicates the stations currently known; the X the stations where the species is extinct; with the triangle the new stations mentioned in this article.

c) *Ecological and phytosociological knowledge about Brassica incana Ten.*

According to literature, *Brassica incana* Ten. is a species usually linked to maritime and calcareous cliffs, from 0 up to 800 m a.s.l. (Snogerup et al. 1990; Pignatti 2018).

In the localities where it has been studied in details, it is linked to a low Mesomediterranean low subhumidbioclimate (Raimondo et al., 1991). It is also known as a Mediterranean chasmophyte, heliophylous, thermophilic and basophilic, generally thriving on nutrient-poor soils sometimes acidic (Raimondo 1997; Pignatti 1982) with slow and constant growth rates that are able to store water and nutrients. In phytosociological studies, the species is indicated as a characteristic species of the rocky vegetation of the alliance *Dianthion rupicolae* Brullo & Marcenò 1979, which brings together the chasmophytic communities, tending to be mesophilic and resistant to marine aerosol (Biondi et al. 2014; Mucina et al. 2016). This alliance belongs to the class *Asplenietea trichomanis* that in Mucina et al. (2016) is described as follows: "chasmophytic vegetation of crevices, rocky ledges and faces of rocky cliffs and walls of Europe, North Africa, Middle East, the Arctic archipelagos and Greenland. The *Asplenietea trichomanis* is a particularly heterogeneous class. These rupicolous plant communities differ not only in species composition, alpha diversity and ecology, but also in overall structure, prevailing life form, and evolutionary history. Especially in the

Mediterranean, the cliff habitats have served as refugia for plants to survive unfavorable climatic conditions as well as grazing pressure. There is a large number of plants exclusive to vertical rock, many of them being regional or local endemics." According to the Italian Prodrome, the class brings together the perennial casmophytic and non-nitrophilous communities that develop in the cracks of rocks, walls and faces (Biondi et al. 2014) sometimes with subalophile species typical of the class *Chritmo maritimi-staticetea* Br.-Bl. in Br.-Bl., Roussine & Nègre 1952 em. Biondi 2007 (Pignatti 2018).

In a phytosociological study carried out in Sicily, focused on the definition of the phyosociological role of *B. incana* (Castellano et al. 2009), the subassassociation *brassicetosum incanae* of the shrubby thermophilous association *Euphorbietum-dendroidis* Guinochet in Guinochet and Drounieau 1944 has been described.

II. Materials and Methods

After a careful examination of all the rocky coastal sites and cliffs of the promontory, from RodiGarganico up to Mattinata, the species has been found only in the ridge of Monte Pucci (northern Gargano), having particular geological conditions that deserved to be investigated with the help of geologists. The integrated approach (botany and geology) has thus made it possible to investigate thoroughly a little-known species, assessed as Data Deficient (DD), according to IUCN criteria (Kell 2011). The study of garganic localities has provided information on the biology of the species and an overview of the ecological conditions (particularly geo-pedological) to which the species is linked. The investigations were carried out in spring/summer 2019.

a) Geological setting and stratigraphic characterization of Monte Pucci area

Monte Pucci area represents a small rocky promontory abutting the Adriatic Sea, delimited by two alluvial plains named Calenella Plain on the west side and Clavia Valley on the eastern side. Along the coast, there are many inaccessible sea-cliffs that allow to observe the stratigraphic succession as well as the loci where *B. incana* has been detected (Fig.2). Along these sea cliffs, instability phenomena are quite frequent and gravity falls accumulate chaotic breccia bodies at the peak of the cliff (Martino & Mazzanti 2014).

Figure 2: The typical stratigraphic succession along the eastern sea-cliff of the Monte Pucci "Trabucco". Decametric olistolith occurs in a very thick breccia layer at the top of the succession. The well bedded intervals represent pelagic deposits interbedded in nummuliticcalciturbidite. Note that the vegetated part is associated to a very thin soil deveopped along some tight steps of the sea-cliff that correspond to thin layer of clay.
Inset: detail of the habitat of *B. incana*.

The Gargano Promontory consists of a thick pile of carbonate rocks formed during the Mesozoic and Cenozoic Periods in different depositional environments of a huge carbonate platform called Apulia Carbonate Platform (ACP) (Bosellini et al. 1999; Borgomano 2000; Morsilli et al. 2004, 2017b; Morsilli 2016). In the Gargano Promontory, contrary to other parts of the Apulia Region, it is possible to observe the transition between the shallow-water inner platform facies and the deep-water basinal successions. Particularly, in the study area of Monte Pucci only basinal facies crops out. Here, the stratigraphy is quite simple and only two stratigraphic units can be detected: the Scaglia Formation (Upper Cretaceous) and Peschici Formation (Middle Eocene) separated by an unconformity that represents a long hiatus of about 40 My (Morsilli et al. 2017a). The Scaglia Formation (from Cenomanian to Santonian p.p.) – consists of thinly bedded (5 to 30 cm) white lime mudstone, generally with layers or nodules of brown to orange cherts. In some outcrops, some breccia or

calciturbidite beds occur related to a coeval submarine gravity-flow. The thickness of this stratigraphic unit is about 90-100 m in this area. This unit was deposited in a relatively deep basin and consists mainly of pelagic accumulation of calcareous nannoplankton and planktonic foraminifera (Bosellini et al. 1999). The Peschici Formation (from Lutetian to Bartonian) - consists of various lithofacies, mainly related to gravity flow processes, deposited in a slope to base of slope setting. The main lithofacies consists of meter-thick coarse calciturbidites rich in large nummulitids, alternating with "chalky" lime mudstones and fine-grained calcarenites. Furthermore, breccia bodies with clasts of shallow-water Eocene (corals) or Cretaceous origin (rudists) are also present. Along the main road climbing to Monte Pucci watching tower from Calenella Plain, the complete stratigraphic succession crops out (Borrelli 2017; Morsilli et al. 2017a).

Along the sea-cliff, re-sedimentation processes are clearly evident, as the decametric well stratified olistolith embedded in the breccia layers that crops out close to the "Trabucco" of Monte Pucci. The unconformity separating the Eocene deposits from the underlying deep-water Cretaceous ScagliaFm is not a transgressive contact, as previously interpreted, but a submarine unconformity onlapped by gravity-displaced and pelagic sediments (Bosellini et al. 1999; Morsilli et al. 2004). Therefore, it is not yet clear which mechanisms were involved in the formation of this erosional contact, but a slump scar or the prolonged activity of deep-sea currents could explain this unconformity (Bosellini et al. 1999).

b) Survey methodology and analysis

The samples were collected in the period of full development of the plant, in which all diagnostic characters are visible; the taxonomic identification was done according to Flora d'Italia (Pignatti1982; Pignatti 2018). The updated checklist of the vascular flora native to Italy was used to assess the Italian distribution range (Bartolucci et al. 2018), while for the European distribution we fallowed Snogerup et al. (1990). The localities of occurrence have been geo-referenced by a GPS (WGS84: Datum). Few intact samples were taken (the species is widely subject to attacks by *Pierisbrassicae*)and collected in the only accessible point of cliff, represented by the edge of the upper terrace of the cliff. A herbarium sheet is stored at the *Herbarium Anconitanum* (ANC) of the Polytechnic University of Marche. All other occurrences of the species on the cliff were detected through UAV (DJI Mavic Pro) with 4k resolution.

In order to assess the participation of the species in habitat of conservation interest according to the Habitats Directive, we consulted the Italian Interpretation Manual of the 92/42/CEE Directive (Ministry of the Environment 2016) while to understand the role of the species in phytosociological terms, we referred to the Vegetation Prodromeof Italy (Biondi et al. 2014). For the bioclimatic classification of the Gargano's localities we followed Pesaresi et al. (2017).

The video analysis permits to reconstruct the stratigraphy and morphological features of the sea-cliffs, as bedding planes and thickness, fracture and occurrence of the main facies of the stratigraphic unit that crops out in this area. Changes in bed thickness, as well as the presence of thin marly beds, create some morphological steps or narrow terraces along the vertical cliffs, where very thin soil horizon can accumulate and retain by vegetation(Fig. 2).

The geological characterization of the Monte Pucci area has been conducted with the traditional methods that include a geological survey and a stratigraphic-sedimentological analysis of the outcropping part of the succession. Rock samples have been collected and studied in thin section under plane-polarized microscope, to characterize the composition, texture and fossil contents.

Due to the occurrence of a single stratigraphic unit, we collected only one soil sample (GPS position), successively analysed at the Department of Physics and Earth Science of the University of Ferrara. The soil sample has been analyzed in terms of texture, composition and geochemical elements.

For the geochemical analysis, after 12 h of heating at 550°C (to remove the organic matter), the sample was powdered in an agate mill and approximately 4 g of powder was hydraulically pressed with boric acid to obtain powder pellets. Simultaneously, a sample aliquot of 0.5–0.6 g was further heated for approximately 12 h in a furnace at 1000 °C to determine the loss on ignition (LOI). This parameter measures the total concentration of volatile substances contained in the sample. The Wavelength Dispersive X-Ray Spectrometry (WDXRF) analysis of the powder pellets was performed using an ARL Advant'X spectrometer Thermo Scientific (Waltham, MA, USA). Calibrations were obtained analysing certified reference materials, and a matrix correction was performed according to the method proposed by Trail and Lachance (1966). Precision and accuracy calculated by repeated analyses of international standards with matrices comparable to the studied samples (Di Giuseppe et al. 2014) were generally better than 3% for Si, Ti, Fe, Ca and K, and 7% for Mg, Al, Mn and Na. For the trace elements (above 10 ppm), the errors were generally better than 10%.

III. Results

In the Gargano Promontory, the species has been found only in two localities occurring in the same complex of cliffs of the Promontory of Monte Pucci, near Vico del Gargano (N41°56.806', E15°59.610'; N41°56.716', E15°59.423'), from 30 to 83 m a.s.l., on

crag (indicated with the triangle in Fig.1). Both localities are north-faced. The habitat of the species is circumscribed to small rocky terraces along steep cliffs exposed to marine aerosol and humid sea currents, through which it satisfies most of its water needs. It forms poor plant communities with other rupicolous species such as *A. commutatum*, *Anthyllis barba-jovis*, *Matthiola incana*, sporadically *Brachypodium retusum*, *Campanula garganica*, and *Capparis rupestris* subsp. *rupestris*. We found some plants on the plans of the cliff on small cones of recent erosion as well. According to the Italian Interpretation Manual of the 92/42/CEE Directive Habitats (Ministry of Environment 2016), the species is a floristic element of the habitat 8210 "Calcareous rocky slopes with chasmophytic vegetation" and specifically of the subtype 62.14 "Community of southern Italy (*Dianthion rupicolae*)".

The main components of soils found in the Monte Pucci sample are mostly related to a siliciclastic composition with abundant Silica and Al_2O_3 and subordinate CaO (Fig.3). The substrate where the soil was formed consists of pure carbonate rock. Only some very thin clay layers interbedded in some part of the succession occur. The soils where the species thrives are a few centimetres thick and they rises from the activity of winds, which deposits fine and inconsistent particles where the plant roots develop.

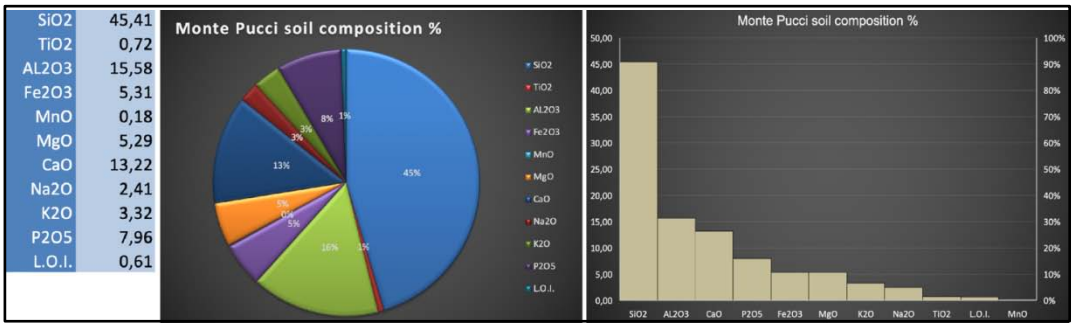

Figure 3: Main components of the Monte Pucci soil

IV. Discussion

As regards the bioclimatic classification, the Gargano localities are part of the Mediterranean macroblioclimate (Pesaresi et al. 2017) like all other localities known in Italy. On the contrary, the localities of occurrence in the Balkan peninsula are mainly classified in the temperate macrobioclimate. The Gargano localities, as well as those in Tuscany and Campania, are part of the lower mesomediterranean bioclimate while the Calabrian and Sicilian ones, are part of the thermomediterranean bioclimatic belt (Syracuse). Therefore, the species grows with different bioclimatological characteristics, ranging from the more mesophile conditions of the temperate macrobioclimate to the warmer ones of the Mediterranean macrobioclimate.

Trace element analysis shows how the sample has abundant Ba, followed by other elements such as Ce, Cr and V with concentration around 100 ppm (Fig.4). It is interesting to note as, contrary to what we expected, the soil composition reveals a very high SiO2 concentration. A possible explanation of this high content in silica can be related to the presence of nearby siliciclastic sand beaches of the Calenella Plain, where very thin sand composed of quarz, K-feldspar and skeletal carbonate shells occur. During wind storms, this very thin sand can be blow out from the beach and transported for a short distance along the sea-cliff and trapped in the vegetation root and incorporate into the soil matrix. Another source of silica can be related to the fore mentioned clay layers, but the inaccessibility of the sea-cliff prevented sampling. The geology of the Gargano Promontory is characterised by the presence of silicon dioxide (SiO2) occurring as oval nodules and/or beds in carbonate rocks: this abundance of silicon dioxide tends to acidify the soils, a condition that explains the presence of typically acidophilic plant species and communities (Biondi et al. 2008).

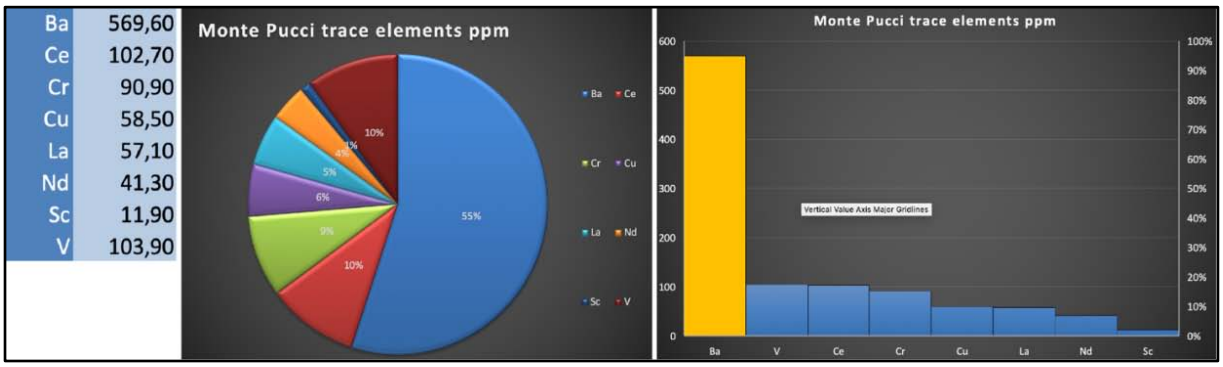

Figure 4: Trace elements concentration of Monte Pucci soil

V. Conclusions

This ecological study of the localities of occurrence of *Brassica incana* in the Gargano promontory has confirmed what was already known for the habitat characteristics of the species, such as its heliophilia (the species is the first one that colonizes small terraces giving rise to small communities), its presence on maritime rocky cliffs and the calcareous nature of the substrates. The localities from Gargano also reveal a link with generally acidic microsoils with incomplete stratigraphy and poorly structured, vertically developed and exposed to the north. They are made up of pure carbonate rock, with marginal presence of very thin clays and high concentrations of SiO2 along with oxides of aluminium (Al2O3) and oxides of calcium (CaO), due to the original stratigraphy that the Gargano presents only in the ridges of Monte Pucci.The investigations on the Gargano, with an integrated approach of study, have revealed that the localized presence of the species is explainable for its link to precise geological units.

The topography of the sitescharacterised by steep cliffs completely inaccessible, has not alloweda detailed surveys of the plant communities yet, thus makinga phytosociological classification impossible.

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