Acta Biologica Turcica, 35(4), D1:1-9, 2022 Received 07 Dec. 2021; Accepted 27 Jan. 2022; Published Online 08 Feb. 2022

ACTA BIOLOGICA TURCICA

E-ISSN: 2458-7893, http://www.actabiologicaturcica.com

Research article

The chemical composition and the ultrastructure of the nest material of *Polistes dominulus* (Christ, 1791) and *Polistes nimpha* (Christ, 1791) (Hymenoptera: Vespidae) in Adana Province (Türkiye)

Filiz ŞEKER MİRAL[1], Nil BAĞRIAÇIK[2,*]
Department of Biology, Faculty of Art and Science, Nigde Ömer Halisdemir University, Nigde, Türkiye
*Corresponding author e-mail: nil@ohu.edu.tr

Abstract: The nest architectures are important for the classification of Vespidae. There are both intraspecific and interspecific differences in nest architecture and its ultrastructure. In this work, the properties of materials used in the nest construction of *Polistes dominulus* (Christ, 1791) and *Polistes nimpha* (Christ, 1791) picked from Adana province (Türkiye) were determined and compared. Although the nests of the two species were architecturally similar, microscopic examination revealed the differences in structural features. The differences in fiber thickness, amount of oral secretion, water holding capacity, amount of oil, and chemical elements of the samples were determined. As a result of elemental analysis, C, O, N were determined as major elements and Si, Ca, Mg, Al, Fe, Cl, and K as minor elements. The differences may be arisen from the choice of nest material by the wasps.

Keywords: Paper wasp, nest material, Adana, Turkey.

Citing: Şeker Miral, F., & Bağrıaçık, N. (2022). The chemical composition and the ultrastructure of the nest material of *Polistes dominulus* (Christ, 1791) and *Polistes nimpha* (Christ, 1791) (Hymenoptera: Vespidae) in Adana Province (Türkiye). *Acta Biologica Turcica,* 35(4), D1: 1-9.

Introduction

The Vespidae are a large, diverse, cosmopolitan family of wasps that includes almost all known eusocial wasps. Nesting behavior and nest architecture are important for both the classification and behavioral biology of the Vespidae (Wenzel, 1998). The Polistinae are known as paper wasps (Reeve, 1991). They produce natural paper pulp using various organic and inorganic materials to build their nests (Spradbery, 1973; Edwards, 1980). They scrape the fibers from plants, vegetables, weathered wood, and other sources and chew on their mouths with the oral secretion (Evans & West Eberhard, 1970; Jeanne, 1975). The oral secretion glues the plant fibers together to protects the nest from the effects of rain (Kudô et al., 2001). *Polistes* build their nests in the form of a single petiole and a comb (Jeanne, 1975). The comb is suspended from the substrate by one or more petioles. The form of combs is typical for a species or a group of species. The combs may be constructed as elongated, pedant, rounded or horizontal in *Polistes* species (Downing & Jeanne, 1986). Nests are built on plants and man-made buildings like roofs and eaves. There are interspecific differences in the selection of nesting places (Evans & West Eberhard, 1970).

The quality of nesting paper depends on the choice of nesting site, the type of organic and inorganic materials. The nest components, the shape of the nest, and the characteristics of the nest area affect the physical features of the nest material (Cole et al., 2001). Nests at aerial sites are constructed of more pliable and waterproof paper than those at subterranean sites or in caves (Matsuura & Yamane, 1990). While species of *Vespula* and *Dolichovespula* build their nests in the air and use sturdy nest paper (Greene, 1991), some species of *Vespula* that build their nests in underground sites have brittle nest paper (MacDonald, 1980). Hornets use mud and soil along with plant fibers as building materials. Some of the magnetic minerals that can be found in the soil around the nest such as Titanium, Zirconium, and Ferritin, are embedded in the comb wall (Ishay et al. 2003, 2008).

Nest materials collected from the environment are chewed by the wasps and mixed with oral secretions. A large part of the dry weight of the nest consists of oral secretion. The secretion is smeared on the surface of the nest by the wasps for protection against rain and weathering (Kudô et al., 1998; Yamane et al., 1998). The salivary secretion is largely composed of protein (Kudô et al., 2000). The protein content in the saliva of *Ropalidia opifex* and *Polistes chinensis* has been estimated to be 90% and 70%, respectively (Maschwitz et al., 1990; Kudô et al., 1998). Alanine, glycine and serine were found to be the main components in the saliva of *R. opifex* (Maschwitz et al., 1990), *Polistes annularis* (Espelie & Himmelbach, 1990) and *Polistes metrius* (Singer et al., 1992). Nitrogen, an important structural component of the nest and found in saliva (Ertürk, 2017), can be used as a measure of the amount of oral secretion (Bagrıaçık, 2011).

Turkey is located in Asia Minor and has a very rich and diverse fauna and flora due to its topographic structures, biogeographic features and macro-microclimatic variations. Although the Vespidae fauna is abundant in Turkey (Yıldırım & Kojima, 1999), the studies on the nesting behavior and the characteristics of nests of Vespidae are limited. Vespid nests from different regions of Turkey were examined in terms of their structural properties. Constructional properties of the nests of *Vespa orientalis* in Niğde, *Vespa crabro* in Zonguldak (Bagrıaçık, 2011); *Polistes gallicus*, *Polistes dominulus*, *Polistes nimpha* in Niğde (Bağrıaçık, 2012); *P. nimpha* collected in different provinces in Central Anatolia (Bağrıaçık, 2013a); *Dolichovespula media* in Niğde, *Dolichovespula sylvestris* in Zonguldak (Bağrıaçık, 2013b); *Dolichovespula saxonica* in Black Sea Region (Ertürk, 2017); *Vespa crabro* and *Polistes dominula* in Trabzon (Ertürk & Bağdatlı, 2018, 2019) were determined.

In this work, the chemical components and ultrastructural characteristics of the nest materials of *Polistes dominulus* and *Polistes nimpha* distributed in Adana province were analyzed by SEM and EDX techniques. The saliva rate, fiber thickness, element composition and absorbency of the nest wall were calculated.

Materials and Methods

Collecting samples

Adana, located in the southeastern part of Turkey, has a Mediterranean-types of climates and vegetation. While the northern part of this province is highland at the foot of Taurus Mountains above 700 m, the eastern part is lowland, the Adana plain, from sea level to 600-700 m. The nests of *P. dominulus* were collected at an altitude between 10-620 m, while the nests of *P. nimpha* were found above 700 m between April and September 2016. The knowledge about the localities of collecting samples is listed in Table 1. The nests were kept in the Niğde Ömer Halisdemir University Biology Department Entomology Laboratory.

Table 1. Collection sites, altitudes, and geographical coordinates (No* refers to sample number).

No*	Locality name	Altitude (m)	Coordinate	Species
1	Karatas	10	36°56'N, 35°38'E	*P. dominulus*
2	Yumurtalık	20	36°81'N, 35°75'E	*P. dominulus*
3	Yüregir, Solaklı	23	36°80'N, 35°33'E	*P. dominulus*
4	Ceyhan, Toktamıs	25	36°99'N, 35°76'E	*P. dominulus*
5	İmamoğlu	84	37°25'N, 35°66'E	*P. dominulus*
6	Cukurova, Topalak	150	37°09'N, 35°27'E	*P. dominulus*
7	Sarıçam, Menekse	160	37°08'N, 35°35'E	*P. dominulus*
8	Kozan, Anavarza	200	37°25'N, 35°90'E	*P. dominulus*
9	Karaisalı	240	35°25'N, 35°05'E	*P. dominulus*
10	Feke, Merkez	620	38°81'N, 35°91'E	*P. dominulus*
11	Aladağ, Kabasakal	700	37°55'N, 35°35'E	*P. nimpha*
12	Pozantı, Eskikonacık	995	32°38'N, 34°86'E	*P. nimpha*
13	Saimbeyli, Gülseren	1050	37°90'N, 36°08'E	*P. nimpha*

Surface Observing

Nest surfaces were observed and monitored using Olympus SZX16 Stereomicroscope and EVO Zeiss 40 Scanning Electron Microscope in Central Laboratory at Niğde Ömer Halisdemir University.

Physicochemical measurable parameters

***Amount of saliva*:** The pieces of dried comb wall were weighed. The pieces were placed in a 0.5 N KOH solution at 70°C for 4-5 hours to dissolve the oral secretions. The fibers were separated by filtration. The fibers washed in water were dried in an electric oven and reweighed with filter paper. Saliva ratio was calculated (Yamane et al., 1998).

Absorbency: The pieces of the outermost wall were weighed. Each piece was immersed in water for 30 seconds and then re-weighed with a precision balance. Absorbency, expressed as a percentage, was estimated (Curtis et al., 2005).

Fiber thickness: The thickness of five randomly selected fibers was measured using Scanning Electron Microscopy (Zeiss Evo 40). The mean and standard error of the thicknesses were reported in Table 2.

Amount of oil: 0.2 g of the nest pieces were weighed. The pieces, which were placed in separate tubes containing ethyl ether, were kept in the tubes for one hour, then extracted, dried and reweighed. The difference between the two measurements showed the amount of fat (Yamane et al., 1998).

Elemental composition: The elemental components of nests was determined using Energy Dispersive X-Ray Analysis (EDX) from SEM (Zeiss Evo 40) in Niğde Ömer Halisdemir University Central Research Laboratory. This X-ray technology is used to identify the essential components of substances. The EDX system is an add-on device to Scanning Electron Microscope. The peaks of the spectra formed during the EDX analysis indicate the constituent elements of the analyzed sample (Fernandez-Segura & Warley, 2008).

Statistics: The relationships between the amount of saliva, water absorption capacity, and fiber thicknesses were analyzed using the Pearson correlation method.

Table 2. Measurable parameters of *Polistes dominulus*' and *Polistes nimpha*' nests

Species	No	Weight of nest (g)	Proportion of saliva (%)	Water Abs. (%)	Amount of oil (g)	Fiber thickness (µm)
P. dominulus	1	4,92	2,23	169	0,15±0,03	10.59±1.45
	2	1,93	1,5	285	0,02±0,04	11,67±0.85
	3	3,14	3,8	251	0,02±0,03	15.2±3.31
	4	9,13	9	67	0,56±0,01	18.11±4.47
	5	4,8	2,29	188	0,02±0,03	13.97±1.35
	6	24,32	1,15	203	0,04±0,03	10.76±1.69
	7	2,93	3,4	313	0,01±0,02	17.92±3.78
	8	1,71	0,58	100	0,03±0,04	13.25±1.89
	9	1,04	12,5	60	0,02±0,01	4.46±1.38
	10	3,58	5,09	182	0,05±0,04	18.52±8.94
P. nimpha	11	11,07	0,5	273	0,08±0,07	12.47±1.92
	12	3,63	2,75	346	0,03±0,04	15.09±4.99
	13	4,85	2,06	100	0,02±0,01	12.6±1.94

Results and Discussion

In this study, morphological and ultrastructural composition of nests belonging to *P. dominulus* and *P. nimpha* collected Adana province were analyzed. All nests except one were collected from open areas close to human buildings. The nest was taken out of the old iron pipe. The morphological examination of the nests of each species showed that the basic structure of the nest architecture was compatible with the literature (Wenzel, 91; Jeanne, 75; Evans & West Eberhard, 1970; Bagrıaçık, 2012). The nests were without an envelope, and suspended with a petiole. Nest sizes were variable (small or medium). The dimensions of the smallest and the largest nest were 2x3,5 cm, and 9,5x13 cm, respectively. Long plant fibers and hairs, inorganic particles, and membranous saliva were monitored in microscopic images of nest surfaces. They were consistent with the images of *P. dominulus'*, *P. gallicus'* and *P. nimpha'* nest surfaces (Bagrıaçık, 2012). *Polistes* species build arboreal nests consisting of one or more petioles and crests without sheaths, sometimes in cavities (Downing & Jeanne, 1986; Wenzel, 1998), and choose long plant fibers and hairs as building material

(Wenzel, 1991). The *Vespa* species collect decomposed wood, the dead parts of living trees, and inorganic material (Matsuura, 1991; Ganor & Ishay, 1992). While *Vespa orientalis* and *Vespa crabro* prefer short woody debris, old rotting chips, and soil; *Dolichovespula* species choose long and woody fibers (Wenzel, 1991; Cole et al., 2001; Bağrıaçık, 2013b; Ertürk, 2017; Ertürk & Bağdatlı, 2019).

After observing the nest surfaces using SEM, it was found that the ultrastructure of the nest wall of *P. dominulus* and *P. nimpha* was similar (Figure 1-2). The plant fibers were fibrous, not lignified. They were disorderly bound and covered with oral secretion, which appeared as thin film in the photographs. Saliva is as a thin film in the nest of *P. dominulus*, *P. gallicus* and *P. nimpha* (Bağrıaçık, 2012). In nest surfaces of *V. orientalis* and *V. crabro* are short and thick fibers with saliva like varnish (Bagrıaçık, 2011). The saliva is like thin varnish beads in the nest surface of *D. saxonica* (Ertürk, 2017). In *D. media*' and *D. sylvestris*' nests, the fibers in the envelopes are more densely organized and tightly adhered than in the combs (Bağrıaçık, 2013b). It was determined that the fiber thicknesses ranged from approximately 2 µm to 32 µm. It was thought that the difference in fiber thickness was caused by the thickness of the plant fibers collected by wasps, and the particles seen on the fibers were soil pieces that were somehow carried to the nest while the wasps were nesting. Some particles may have been mixed in with the plant fibers from the environment.

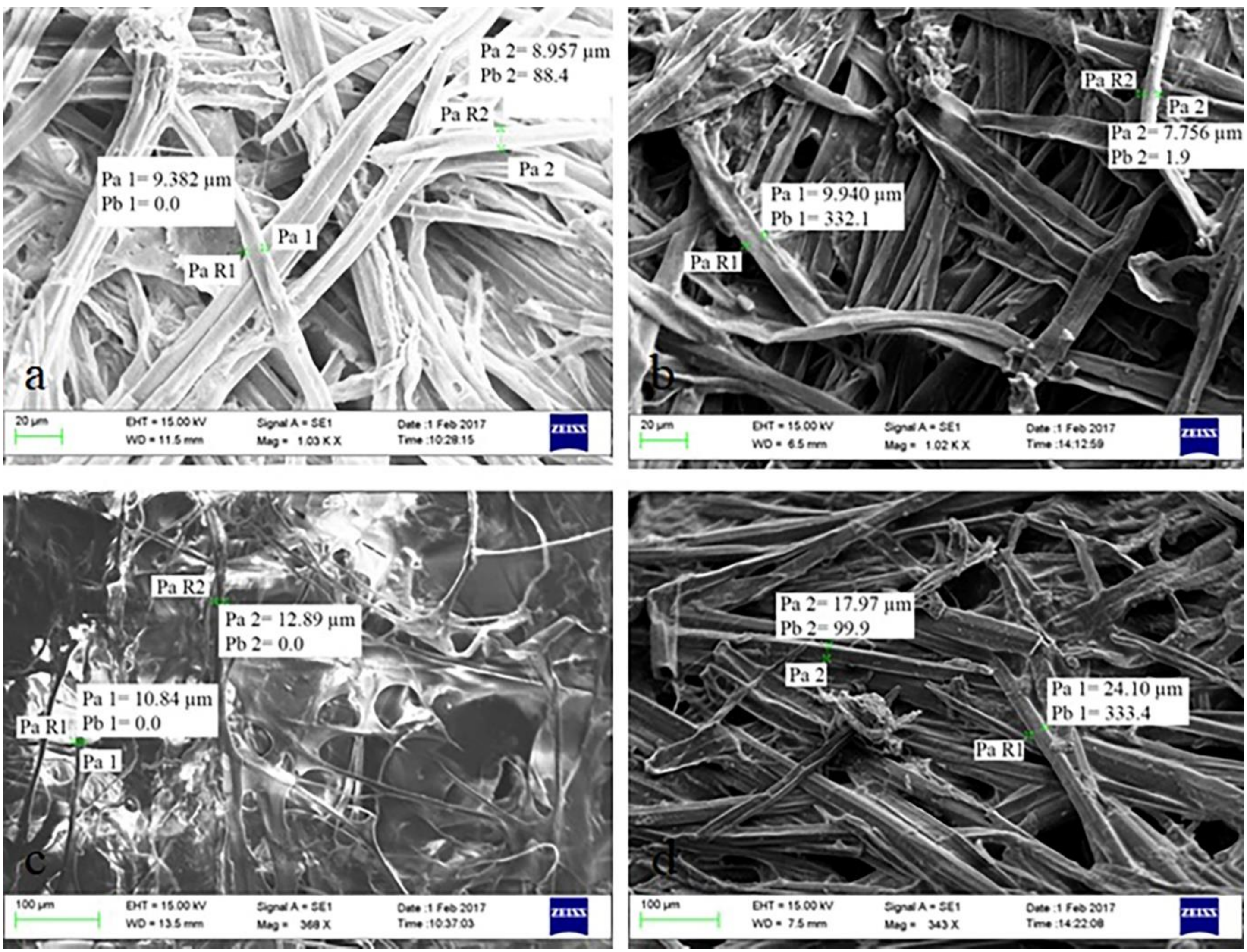

Figure 1. Ultrastructure of the nest's wall of *P. dominulus* by SEM, and some measurements of fiber thicknesses.

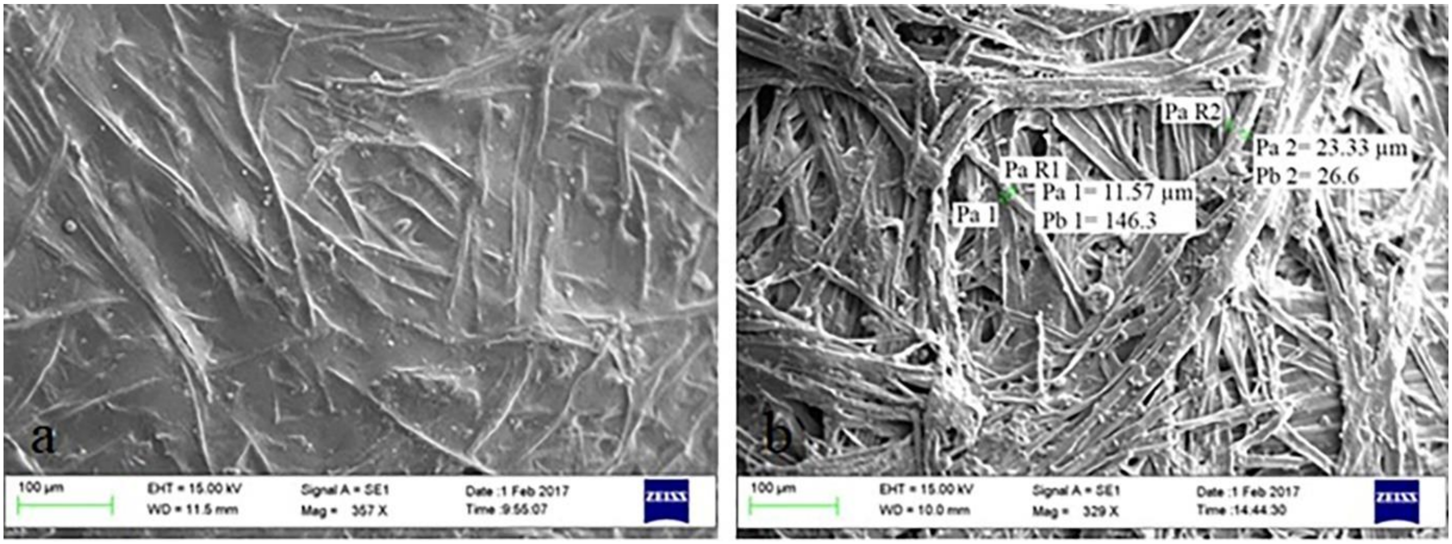

Figure 2. Ultrastructure of the nest's wall of *P. nimpha* by SEM, and some measurements of fiber thicknesses.

All measurable parameters (weight of nest, proportion of saliva, water absorption capacity, amount of oil, fiber thickness) were reported in Table 2. The mean value of nest weight, saliva percentage, and water holding capacity of *P. dominulus* and *P. nimpha* were calculated as 5.79± 6.91g, 4.15±3.81%, 181±86.92% and 6.51±3.99g, 1.77±1.55%, 239±126%, respectively. The minimum and maximum thickness in the nests of *P. dominulus* were 2.43-31.65µm, and in the nests of *P. nimpha* were 10.42-23.33 µm in this study. Fiber thicknesses were measured as 8.7 µm, 5.73 µm, 9.04 µm in *P. dominulus*, *P. gallicus* and *P. nimpha* (Bağrıaçık, 2012), 7.41-18.87 µm in *V. orientalis*, and 6.31-17.89 µm in *V. crabro* (Bağrıçak, 2011), and 8.09-11.21 µm in *P. nimpha* (Bağrıçak, 2013a).

Water absorption capacity is related to the type of plant fiber and the amount of saliva. There is a correlation between water holding capacity and the low humidity of the nest (Biermann, 1993). The nests of *P. dominulus* are more absorptive than those of *P. fuscatus*, as the correlation between the protein concentration and the absorbent capacity of the nest of *Polistes dominulus* was positive, while it was negative for *P. fuscatus* (Curtis et al., 2005). The water absorption capacities were calculated to be 110-140% for *D. saxonica* (Erturk, 2017), 217-764% for *P. nimpha* (Bağrıaçık, 2013a), 91% for *V. orientalis* and 100% for *V. crabro* (Bağrıaçık, 2011), 100% for *P. dominula* and 53.19% for *V. crabro* (Ertürk & Bagdatli, 2019). In this study, the water holding capacities of *P. dominulus* and *P. nimpha* are close to the previous data. The amount of saliva and low humidity of the fibers can have an effect on absorbency. According to Pearson's correlation method, there was a negative relationship between the amount of saliva and water holding capacity (Pearson Cor = -0.510, p 0.05) and a positive correlation between water absorbency and fiber thicknesses (Pearson Cor = 0.308, p 0.05) in the nests of *P. dominulus* and *P. nimpha* (n=13). It can be said that as the amount of saliva increases, the water absorption capacity decreases, and as the fiber thickness increases, the water absorption capacity also increases.

The mean amount of oil and oily matter in *P. dominulus* and *P. nimpha* nests as organic compounds were measured as 0.09±0.16g and 0.04±0.03g, respectively. In this study, the amount of oil in the nest papers of two species was lower than in the nests of other species. The organic components of the nest were found to contain 5.43% oil in *P. dominula*, 2.48% in *V. crabro* (Erturk & Bagdatli, 2019) and 12-18% in *D. saxonica* (Erturk, 2017). Hexadecanoic acid and octadecenoic acid were found on the nest paper of *P. annularis* (Espelie & Hermann, 1990).

According to EDX analysis, carbon, oxygen, and nitrogen were the main components of the nests. The amount of nitrogen was less than that of carbon and oxygen in all nests. In this study, the elemental composition of *P. dominulus* and *P. nimpha* was similar. The mean values of the atomic fraction of C, O, and N in the nest wall of *P. dominulus* and *P. nimpha* were calculated as 38.91±17.11, 42.59±8.23, 14.28±5.2 and 52.91±11.46, 28.7±6.52, 17.35±5.28, respectively. Calcium, silicon, aluminum, iron, potassium, chloride and magnesium were minor components of the nests but they were not found in all the nests. The composition and atomic proportion of the elements were given in Table 3, and the spectra of the elements are shown in Figure 3. In the nest paper of *P. nimpha*, *P. gallicus*, *P. dominulus*, *V. crabro*, *V. orientalis*, *D. saxonica*, *D. sylvestris* and *D. media* C, O, N were the major elements and Ca, Si, Al, Fe, K, Cl, Mg were the minor components (Bağrıaçık, 2013a, 2013b, 2012, 2011; Erturk, 2017; Ertürk & Bagdatli, 2019). The oral secretion of *Polistes sp*. consists of protein (Kudô et al., 2001, Espelie and Himmelbach, 1990; Singer et al., 1992; Kudô, 2000). Since *V. orientalis* collects more soil or mud than *V. crabro*, the amount of silicon is higher than other elements in the nest of both species (Bagrıaçık, 2011). Inorganic materials were never detected in the nest of *Polistes chinensis* (Kudô et al., 1998) and *P. riparius* (Yamane et al., 1998). *Polybia paulista* collects mud or/and inorganic particles as nest material (Kudô et al., 2001). The source of inorganic particles in the *Polistes* nests may be accidental mixing when collecting plant fibers.

Saliva rate was calculated as min. 5% and max. 9%. The percentage of saliva in this study is very low compared to the results of other studies. Wasp chew and agglutinate plant fibers and smear the nest surface with saliva. Nitrogen is a marker used to determine the amount of saliva (Kudô et al., 1998). The ratio of saliva and nitrogen in *Polistes* nests varies greatly (Espelie & Himmelbach, 1990; Singer et al., 1992). The proportion of oral secretion was 52-57% in *Polistes riparius*, 60-65% in *P chinensis* (Yamane et al., 1998); 39-63% in *P. nimpha* (Bağrıaçık, 2013a), 58% in *P. gallicus*, 23% in *P. gallicus,* 22% in *P. dominulus* (Bağrıaçık, 2012); 44.44% in *P. dominula*, 41.05% in *V. crabro* (Erturk & Bagdatli, 2019), 20% in *V. orientalis*, 77% in *V. crabro* (Bagrıaçık, 2011). The nitrogen content is very low, as *Polybia paulista* uses small amounts of oral secretion. (1.59-2.14%) (Kudô et al., 2001). The nitrogen concentrations of *Polistes fuscatus* (McGovern et al., 1988), *P. annularis* (Espelie and Himmelsbach, 1990) and *Polistes metricus* (Singer et al., 1992), *D. saxonica* (Erturk, 2017) were 6.6%, 2.8%, 1.4-8.0% and 0.61%, respectively. The nitrogen content in the nest of *Vespa analis* was 1.1-2.0%, *Vespa simillima* 0.9-2.0% and *Vespa crabro* 2.5% (Kudô et al., 2001). The nesting paper of *P. annularis* is predominantly cellulose (Espelie & Himmelsbach, 1990). The saliva on the comb wall of *D. media* and *D. sylvestris* were richer than that on the envelope (Bağrıaçık, 2013b).

In this study, chemical and ultrastructural features were determined in the nest paper of *Polistes dominulus* and *Polistes nimpha* in Adana. Thermal state of the nest can play an important role in nest construction. The microclimate of the nest area and the honeycomb insulation affect the thermal condition of the nest (Yamane et al., 1998). Differences in nest materials may be due to nest site selection and environmental factors (precipitation, humidity, vegetation, temperature, etc.). Species-specific variation in nest site and type of nest material was estimated in terms of chemical composition and ultrastructural characteristics.

Table 3. Composition and atomic % of elements by EDX analyze in *Polistes dominulus*' and *Polistes nimpha*' nests.

	Atomic %												
	Polistes dominulus									*Polistes nimpha*			
Elements	Karataş	Yumurtalık	Yüreğir	Ceyhan	İmamoğlu	Çukurova	Sarıçam	Kozan	Karaisalı	Feke	Aladağ	Pozantı	Saimbeyli
C	18,59	57,43	32,85	52,65	48,81	21,22	41	28,51	42,15	46,2	61,02	47.05	44,8
O	50,57	30,98	52,03	36,14	37,54	56	35,19	46,21	40,17	41,16	24,09	10.49	33,32
N	21,65	10,49	12,78	10,16	12,66	9,01	22,11	19,57	15,88	8,49	13,62	39.84	21,09
Ca	1,22	0,59	-	0,2	0,36	2,13	0,41	1,72	0,6	0,25	0,28	0.23	0,24
Si	0,06	0,07	1,2	0,39	0,13	-	0,56	0,06	0,29	2,21	-	1.47	0,05
Al	-	-	-	0,15	0,06	7,75	-	-	-	0,58	-	0.07	0,07
Fe	1,66	-	-	-	-	-	-	0,89	-	3,37	0,95	-	0,43
K	1,71	0,44	1,14	0,31	0,05	1,41	0,59	0,56	0,37	0,22	0,04	0.86	-
Cu	-	-	-	-	-	-	-	-	0,38	-	-	-	-
Cl	4,54	-	-	-	-	-	0,14	-	0,16	-	-	-	-
Mg	-	-	-	-	0,39	2,48	-	-	-	-	-	-	-

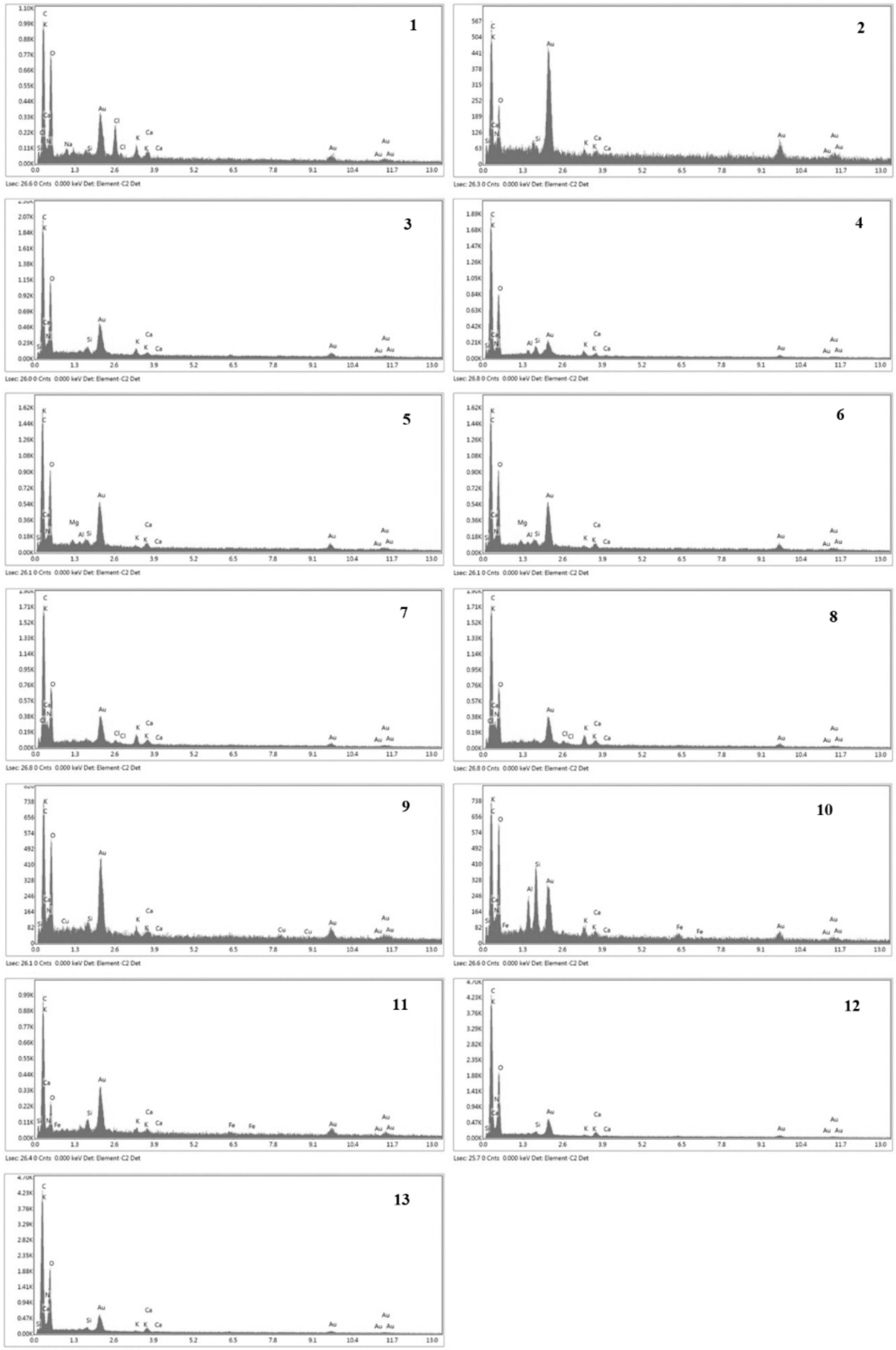

Figure 3. EDX Spectra of nest samples according to locality number.

Acknowledgement

This study was carried out within the scope of the master's thesis accepted in 2018 by Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals followed.

Funding Statement

The authors do not declare any fund.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Bagrıaçık, N. (2011). Determination of some structural features of the nest paper of *Vespa orientalis* Linneaus 1771 and *Vespa crabro* Linneaus, 1758 (Hymenoptera: Vespinae) in Turkey. *Archives of Biological Sciences*, 63(2), 449-455.

Bagrıaçık, N. (2012). Comparison of the nest materials of *Polistes gallicus* (L.), *Polistes dominulus* (Christ) and *Polistes nimpha* (Christ) (Hymenoptera: Vespidae). *Archives Biological Sciences Belgrade,* 64 (3), 1079-1084.

Bağrıaçık, N. (2013a). Some structural features of nest materials of *Polistes nimpha* (Christ, 1791) in several ecological conditions (Hymenoptera: Vespidae). *Journal of the Entomological Research Society*, 15(3), 1-7.

Bağrıaçık, N. (2013b). Some structural features of the nest materials of *Dolichovespula Sylvestris* (Scopoli, 1763) and *Dolichovespula media* (Retzius, 1783) (Hymenoptera: Vespidae). *Journal of Selcuk University Natural and Applied Science*, 1(1), 893-902.

Biermann, C. J. (1993). *Essentials of Pulping and Papermaking*. Academic Press Limited, London.

Cole, M. R., Hansell, M. H., & Seath, C. J. 2001. A quantitative study of the physical properties of nest paper in three species of Vespine wasps (Hymenoptera, Vespidae). *Insectes Sociaux*, 48(1), 33-39.

Curtis, T. R., Aponte, Y., & Stamp, N. E. (2005). Nest paper absorbency, toughness and protein concentration of a native vs. invasive social wasp. *Journal of Chemical Ecology*, 31(5), 1089-1100.

Downing, H., & Jeanne, R. L. (1986). Intra- and interspecific variation in nest architecture in the paper wasp Polistes (Hymenoptera, Vespidae). *Insectes Sociaux*, 33, 422-443

Edwards, R. (1980). *Social Wasps. Their Biology and Control*. Rentokil, East Grinstead, UK.

Erturk, Ö. (2017). Determination of some structural features of the nest paper materials of *Dolichovespula saxonica* Fabricius, 1793 (Hymenoptera: Vespinae) in Turkey. *Entomological Research*, 47(5), 286-294.

Ertürk, Ö., & Bağdatlı, E. (2018). Investigations on morphology of the European Hornet (Vespa crabro) nest and biological development situations of the larvae. *Acta Biologica Turcica*, 31(2), 36-41,

Erturk, O., & Bagdatli E. (2019). Comprehensive study on nest materials of *Vespa crabro* and *Polistes dominula*: Chemical properties and biological characterization with antioxidant and antimicrobial activity. *Biologia*, 74, 797–812.

Espelie, K. E., & Hermann, H. R. (1990). Surface lipids of the social wasp *Polistes annularis* (L.) and its nest and nest pedicel. *Journal of Chemical Ecology*, 16(12), 1841–1852.

Espelie, K. E., & Himmelsbach, D. S. (1990). Characterization of pedicel, paper and larval silk from nest of *Polistes annularis* (L.). *Journal of Chemical Ecology*, 16(12), 3467-3477.

Evans H.E., & West Eberhard M.J. (1970). *The Wasps*. Michigan University Press, Michigan, USA.

Fernandez-Segura, E., & Warley, A. (2008). Chapter 2 Electron Probe X-ray Microanalysis for the Study of Cell Physiology. In: T.D. Allen (Ed.), *Introduction to Electron Microscopy for Biologists*, Vol. 88, (pp.19-43), Academic Press, USA.

Ganor, E., & Ishay, J. S. (1992). The cement in hornet combs. *Journal of Ethology*, 10, 31-39

Greene, A. (1991). Dolichovespula and Vespula. In: Ross K.G. and R.W.Matthews (Eds.), *The Social Biology of Wasps*, (pp.263-304). Cornell Univ. Pres, Ithaca.

Ishay, J.S., Barkay, Z., Eliaz, N., Plotkin, M., Volynchik, S., & Bergman, D. J. (2008). Gravity orientation in social wasp comb cells (Vespinae) and the possible role of embedded minerals. *Naturwissenschaften*, 95, 333–342

Ishay, J. S., Riabinin, K., Kozhevnikov, M., van der Want, H., & Stokroos, I. (2003). A Keystone-Like Grain in Hornet Comb Cells: Its Nature and Physical Properties. *Biomacromolecules*, 4(3), 649-656.

Jeanne, R. L. (1975). The adaptiveness of social wasp nest architecture. *The Quarterly Review of Biology*, 50(3), 267-287.

Kudô, K. (2000). Variable investments in nest and worker product on by the foundresses of *Polistes chinensis* (Hymenoptera: Vespidae). *Journal of Ethology*, 18(1), 37-41.

Kudô, K., Hozumi, S., Yamamoto, H., & Yamane, S. (2000). Amino acid composition of the protein in preemergence nests of *Polistes* (*Polistes*) *riparius*, and its similarity to the consubgeneric wasp, *P.* (*P.*) *chinensis* (Hymenoptera: Vespidae). *Journal of Ethology*, 18(1), 75-77.

Kudô, K., Yamane, S., & Yamamoto, H. (1998). Physiological ecology of nest construction and protein flow in preemergence colonies of *Polistes chinensis* (Hymenoptera: Vespidae): Effects of rainfall and microclimates. *Ethology Ecology & Evolution*, 10, 171-183.

Kudô, K., Yamane, S. O., Mateus, S., Tsuchida, K., Ito, Y., Miyano, S., Yamamoto, H., & Zucchi, R. (2001). Nest materials and some chemical characteristics of nests of a New World swarm founding polistinae wasp *Polybia paulista* (Hymenoptera: Vespidae). *Ethology Ecology & Evolution*, 13, 351-360.

MacDonald, J. F. (1980). *Biology, Recognition, Medical Importance and Control of Indiana Social Wasps*. Purdue University Press, Indiana, USA.

Maschwitz, U., Dorow, W. H. O., & Botz, T. (1990). Chemical composition of the nest walls, and nesting behaviour, of *Ropalidia (Icarielia) opifex* van der Vecht, 1962 (Hymenoptera: Vespidae), a Southeast Asian social wasp with translucent nest. *Journal of Natural History*, 24, 1311-1319.

Matsuura, M. (1991). Vespa and Provespa. In: Ross K.G. and Matthews R.W. (Eds.), *The social biology of wasps*. (pp. 232-262). Cornell Univ. Press, Ithaca.

Matsuura, M., & Yamane S. K. (1990). *Biology of Vespinae Wasps*. Springer Verlag, Berlin.

McGovern, J. N., Jeanne, R. L., & Effland, M. J. (1988). The nature of wasp nest paper. *Tappi Journal*, 71, 133-139.

Reeve, H. K. (1991). Polistes. In: K.G. Ross and R.W.Matthews (Eds.), *The Social Biology of Wasps*. (pp. 99-148). Cornell University Pres, USA.

Singer, T. L., Espelie, K. E., & Himmelsbach, D. S. (1992). Ultrastructural and chemical examination of paper and pedicel from laboratory and field nests of the social wasp *Polistes metricus* Say. *Journal of Chemical Ecology*, 18 (1), 77-86.

Spradbery, J. V. (1973). *Wasps. An account of the biology and natural history of social and solitary wasps*. Sidgwick and Jackson Pres, London.

Wenzel, J. W. (1991). Evolution of Nest Architecture. In: K.G. Ross and R.W.Matthews (Eds.), *The Social Biology of Wasps*. (pp. 480-519). Cornell Univesity Press, USA.

Wenzel, J. W. (1998). *A Generic Key to The Nests of Hornets, Yellowjackets, and Paper Wasps Worldwide (Vespidae, Vespinae, Polistinae)*. American Museum Novitates, USA.

Yamane, S., Kudô, K., Tajima, T., Nihon'yanagi, K., Shinoda, M., Saito, K., & Yamamoto, H. (1998). Comparison of investment in nest construction by the foundresses of consubgeneric Polistes wasps, *P. riparius* and *P. chinensis* (Hymenoptera, Vespidae). *Journal of Ethology*, 16(2), 97-104.

Yildirim, E., & Kojima, J. (1999). Distributional checklist of the family Vespidae (Insecta: Hymenoptera: Aculeata) of Turkey. *Natural History of Bulletin Ibaraki University*, 3, 19-50.