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Croatian diatomites and their possible application as a natural insecticide

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Abstract

In recent decades, there has been an increase in the use of diatomaceous earth (DE) as a natural insecticide because of its low mammalian toxicity, worker safety, low risk of food residues and the occurrence of resistant insect populations associated with the use of chemical insecticides. Therefore there is potential for research into known but previously undescribed Croatian mid-Miocene marine diatomites from the perspective of their potential as proper DE that could be mixed with plant extracts as a new formulation for grain storage protection. The marine diatomites belong to the Paratethyan near shore environment, deposited in the upwelling zone during a mid-Miocene temperate climate. Palaeontological, mineral and geochemical analyses were done on ten promising marly sediments from 26 outcrops and one borehole from the North Croatian Basin. The most important ingredient of diatomaceous sediments is silica (biogenic opal-A and SiO_2 bound in other silicate minerals including quartz, clay minerals, micas, etc.). The amorphous silica content of the tested Croatian diatomites is relatively low (≤ 50%) in comparison with the Celatom® MN 51 standard (medium to high efficient DE) (73.6%), nevertheless they show in some part even slightly better efficacy against insects. It seems that the enhanced content of smectite in diatomaceous sediments also influences increased absorption of DE. Based on palaeontological results, the most efficient diatomites from the Podsusedsko Dolje and Markuševec (Medvednica Mt.) consist of the mid-sized planktonic *Coscinodiscus* group of species where *Thalassionema nitzschioides* dominate and is positively correlated with their absorption. The usage of Boströms' standard formula for getting opal-A from geochemical data was abandoned because of negative results and the modified Murdmaas' formula for hemipelagic sediments was applied. Preliminary results on the aforementioned diatomite (as inert dusts) show good efficacy against tested insects *Sitophilus oryzae* (LINNAEUS), *Tribolium castaneum* (HERBST) and *Rhyzopertha dominica* (FABRICIUS).

Keywords: diatomite, palaeontology, mineralogy, geochemistry, natural insecticide, mid-Miocene, Croatia

1. INTRODUCTION

Diatomite is a porous, lightweight, siliceous sedimentary rock mainly composed of diatom skeletons formed in marine to freshwater environments of mid to high latitudes and contains >30% amorphous opaline silica ($SiO_2{\cdot}nH_2O$). Commercial diatomite contains >60% of biogenic silica (BSi). Mostly high-grade diatomite (>80% BSi) is used as diatomaceous earth (DE), crushed and oven-dried inert dust with a minor content of calcium carbonate, volcanic glass and terrigenous particles (HARWOOD, 2010). Sediments that contains <30% of diatoms belongs to the tripolite.

Biogenic silica is non toxic to mammals and has been registered in many countries as a food additive. DE is known as a potentially useful grain protectant because it is safe to use, does not affect grain end-use quality, provides long-term protection and is comparable in cost to other methods of grain protection (KORUNIĆ, 2013). Ideally, active DE should have a high BSi content with a uniform particle size (less than 10 microns), a high oil sorption capacity, a large active surface, and very little clay and other impurities (KORUNIĆ, 1998). Lately, there are numerous reports from around the world on the widespread resistance of several stored-product insects to grain protectants from the groups of synthetic pyrethroids, organophosphates, carbonates, chlorinated hydrocarbons, *Bacilus thuringiensis,* botanicals and chemicals (SUBRAMANYAM & HAGSTRUM, 1995; KLJAJIĆ & PERIĆ, 2005). Since only a physical method of controlling insects is involved in DE, genetic resistance is generally unlikely according to EBELING (1971). However, a potential tolerance or even resistance should be considered when choosing DE for replacing existing grain protectants (KORUNIĆ, 1998; RIGAUX et al., 2001; FIELDS, 2003; VAYIAS et al., 2008).

The efficacy of DE against insects depends on the different properties of the diatom particles, including physical and morphological characteristics of diatoms, rather than on its origin. The physical property of diatomite is in its specific gravity (density) of diatom frustules (skeletons), which is about half that of water. The perforations (pores and striae) and open structure of diatom frustules renders diatomite a considerably lower density (0.12–0.25 g/cm^3) and high porosity (75–85%), able to absorb and hold up to 3.5 times its own weight in liquid i.e. in this case wax. Well-known diatoms with pores (areolas) have the physical ability to absorb wax (lipids) from the insects' epicuticle, causing its dehydration and death (EBELING, 1971; KORUNIĆ, 1998), according to high absorbency which speeds up the incorporation of oil.

The earliest known Croatian diatomites are from the Podsusedsko Dolje outcrops (Medvednica Mt.), and can be described as diatomaceous tripolite (outcrops J1-J4: PANTOCSEK, 1903

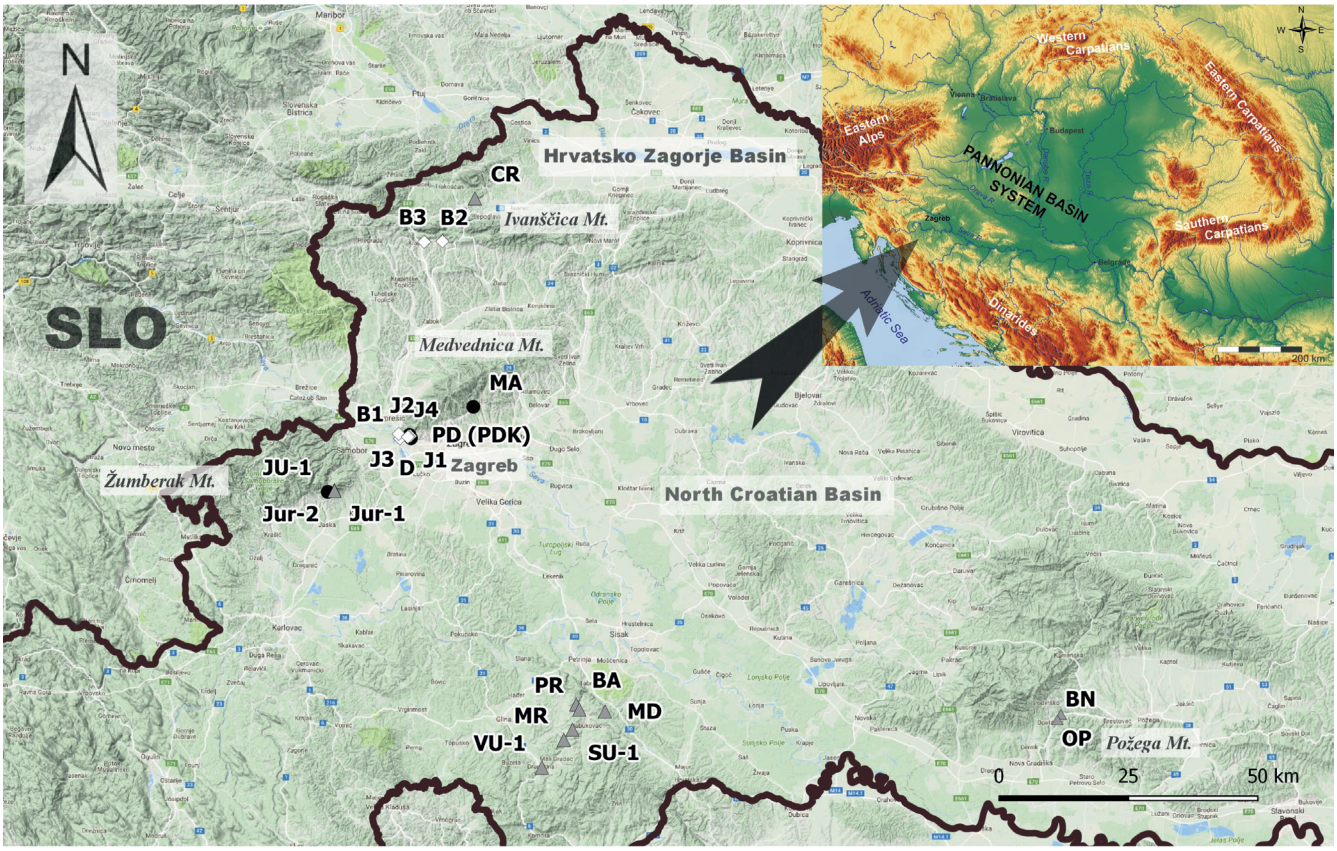

Figure 1. Locations of investigated diatomites in Croatia, Pannonian Basin System based on http://maps-for-free.com: ◇ – J1-J4 (1903 to 1957) and B1-B3 (1981 to 1984); ● – Basic Geological Map of Republic of Croatia 1:50.000 from 2000 to 2009; ▲ – this investigations with explanations in the text.

and JURILJ, 1957; outcrop B1: KOCHANSKY-DEVIDÉ & BAJRAKTAREVIĆ, 1981; outcrops PD-MA: GALOVIĆ & BAJRAKTAREVIĆ, 2006), and Lopatice and Gornja Šemnica in Hrvatsko Zagorje (outcrops B2-B3: BAJRAKTAREVIĆ, 1984) (Fig. 1). Tripolite is a broad term, used in Croatia for laminated marly sediments by many of the aforementioned authors. The first applied terminology of diatomite was used by GORJANOVIĆ-KRAMBERGER (1908) and JURILJ (1957). During the investigations for the Croatian Basic Geological Map 1:50.000 from 2000 to 2004, a few more outcrops of diatomaceous tripolite (i.e. diatomite) were discovered in Hrvatsko Zagorje (CR), at Medvednica, Žumberak (Jur-1 and Jur-2) and Požeška gora Mts. Seventy-seven more diatom taxa were determined during those investigations on the Podsusedsko Dolje outcrops, where 23 were hitherto not known for the Sarmatian (GALOVIĆ & BAJRAKTAREVIĆ, 2006).

Our investigations are part of the Croatian Science Foundation (HRZZ) project „Development of new natural insecticide formulations based on inert dusts and botanicals to replace synthetic, conventional insecticides“ – DIACROMIXPEST conducted from 2014-2016 (ROZMAN et al., 2015). Throughout this research six more outcrops of diatomite have been discovered in the Banovina region, two in Slavonia and one at Žumberak Mt. (Fig. 1). The palaeontological, mineralogical and geochemical results of Croatian diatomites are presented here with their potential usage as DE in agriculture for the protection of stored products.

2. GEOLOGICAL SETTING

The investigated mid-Miocene (Sarmatian) sediments of the North Croatian Basin (NCB) and Hrvatsko Zagorje Basin (HZB), belong to the south-western marginal part of the Pannonian Basin System (PBS) (Central Paratethys) (PAVELIĆ, 2001; PAVELIĆ et. al., 2003) (Fig. 1). This intracontinental region began to develop in the early Miocene as a result of the African and European plate collision (HORVÁTH & ROYDEN, 1981). PBS is surrounded by the Alps, Dinarides and Carpathian Mountains. The evolution of the Central Paratethys was controlled by regional tectonic events, sea-level changes and connections with the Mediterranean, Boreal and Indo-Pacific Oceans (STEININGER et al., 1988; RÖGL, 1996). These connections have been repeatedly disrupted and re-established until the end of the Badenian when the sea level dropped, causing the beginning of the isolation and disintegration of the Paratethys. At the beginning of the Sarmatian, the connection with the other marine areas further weakened, and was followed by isolation; a stronger terrestrial influence favoured further decrease in salinity, resulting in changes to flora and fauna and their endemism. This was all more strongly influenced by climate changes. From the warmer period in the Badenian, the climate during the Sarmatian becomes more moderate with seasonal oscillations (GALOVIĆ, 2001). In the Sarmatian, the connections with the Mediterranean and Indo-Pacific realms also existed, as shown by the last Miocene transgression (RÖGL, 1998; KOVÁČ et al., 2001), and corroborated by investigations of siliceous microplankton (JURILJ, 1957; HAJÓS, 1986; GALOVIĆ & BAJRAKTAREVIĆ, 2006). The total thickness of the Sarmatian deposits is relatively small (50 to 150 m), they predominantly consist of marly sediments of the tripolite type (BAJRAKTAREVIĆ et al., 1986) with rare limestones, clastic and volcanic fragments. Such a small thickness is probably a consequence of the basin deepening (due to lithosphere cooling and compression at the end of the Sarmatian) and less sediment input, which is characteristic of the early post-rifting phase (PAVELIĆ et al., 2003).

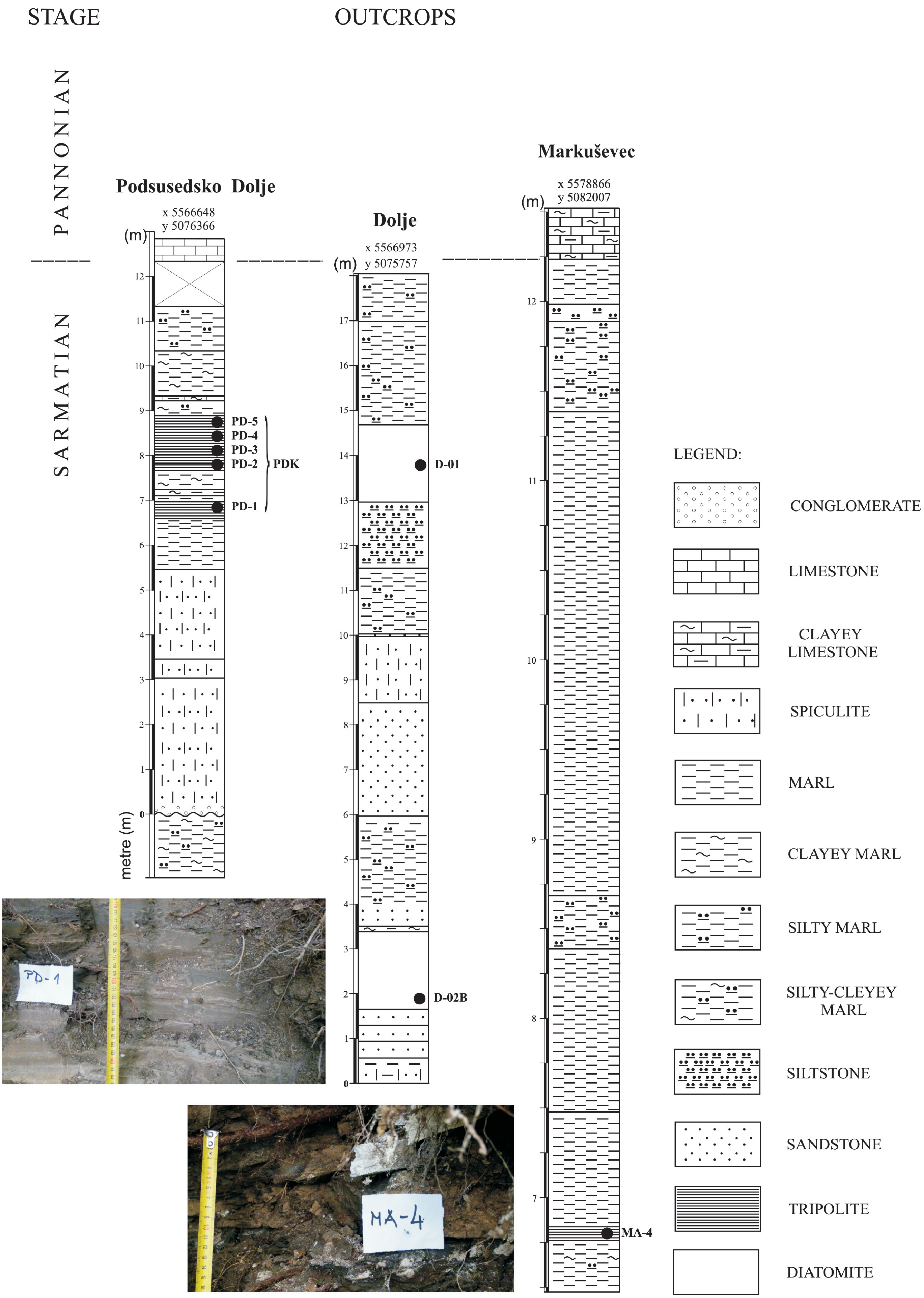

Figure 2. The parts of the geological outcrops from Medvednica Mt. where the most effective Croatian diatomite and tripolite were found.

Since the beginning of the Miocene (23 Ma; OGG et al., 2016), the most commercially exploited diatomaceous earth deposits are lacustrine in origin, although the largest producing deposit that outcrops near Lompoc in California is a marine deposit of Miocene age (DOLLEY & MOYLE, 2003). Nevertheless, other diatomites from Paratethys are also marine deposits of Miocene age from Slovenia, Austria, Czech, Slovakia, Hungary and Serbia (ŘEHÁKOVÁ, 1977; HAJÓS, 1986; HORVAT, 2004; ROETZEL et al., 2006; SCHÜTZ et al., 2007; ROJHT et al., 2010). Well known Croatian diatomites from Medvednica Mt. belong to near shore mid-Sarmatian marine sediments characteristic of the upwelling zone (GALOVIĆ & BAJRAKTAREVIĆ, 2006).

Individual parts of the NCB were uplifted and thus became source regions for clastic Sarmatian deposits, accumulating in environments of reduced salinity (VRSALJKO, 1999). Through advanced ingression and deepening of the depositional environment, thin-bedded to laminated marls and sandstones accumulated (VRSALJKO et al., 2005; VRSALJKO, 1997).

In the NCB Sarmatian shallow-water gravels, biocalcarenites, and limestones were laid down, whereas later laminated marly deposits predominated, due to the deepening of the basin. For the region of the Žumberak and Samobor Mts., Sarmatian sediments consisting of calcite-rich marls and limestones with sandstone intercalations, crop out in a narrow zone along the Badenian deposits, (VRSALJKO, 2003).

3. MATERIALS AND METHODS

3.1. Field research with laboratory tests

The representative sediments for diatom analyses belong to marl, tripolite, laminated marls, diatomite, varve, silt, clay and their varieties (Fig. 2). The thicknesses of the sampled diatomites are small, approx. 48 cm. They are mostly laminated as a result of seasonality. Among 26 well known Sarmatian outcrops of diatomites in Croatia, twelve were selected for further investigation: Baničevac (BN) and Opatovac (OP) in Slavonia, Jurjevčani (JU) at Žumberak Mt., Crkovec (CR) in Hrvatsko Zagorje, Markuševec (MA) and Podsusedsko Dolje (PD) at Medvednica Mt. and Bačuga (BA), Martinovići (MR), Prnjavor Čuntički (PR), Šušnjar (SU), Mali Deanovići (MD) and Vukičevići (VU) in Banovina. Samples from the Dolje borehole at Podsusedsko Dolje were also examined (D) (ROZMAN et al., 2016) (Fig.1**).** In total, 57 samples (each of ca. 10 kg) were taken for preliminary testing on their insecticidal efficacy by comparing them with standard medium to high effective Celatom® MN 51. After testing, ten samples showed promising insecticidal efficacy and were selected for further analyses: MA-4, PD-1, PDK, D-01 and D-02B, JU-1, OP-4 and OP-4A (less laminated marls from the same horizont), MR10 (siltic marl) and MR-10B (mm laminated marl) and examined as natural pesticide (LIŠKA et al., 2015). Sample PDK is mixture of all five PD samples, but milled to a diameter <45 µm for testing efficacy with smaller particles in the second year of the research project. Based on their preliminary physico-chemical properties (palaeontological, mineral and geochemical analyses) only samples from Medvednica Mt. (Fig. 2) were affirmed as diatomite.

3.2. Palaeontology

The standard preparation method used by the Croatian Geological Survey is applied. Approximately 1 cm^3 of the sediment is placed in a beaker and treated with 30% concentrated hydrogen peroxide (H_2O_2) to oxidize organic matter for light microscope (LM) analyses. Siliceous microfossil slides for SEM preparations also include treatment with 20 % of concentrated HCl acid to remove carbonate and 10 % of sodium pyrophosphate to remove clay components. The sediments are then rinsed with distilled water. Some of the samples were treated in an ultrasonic bath for approximately 15 seconds to improve disaggregation. Standard smear slides and SEM stub preparation techniques were used in slide preparation for both LM and SEM analysis. The specimens were coated with gold and studied with a JEOL JSM-35CF SEM. Smear slides were examined using a BH2 Olympus LM.

Composition and diversity of diatom species together with their morphometric analyses are essential for the determination of DE quality. Quantitative methods were undertaken for 300 valves of diatom genera along transects under 500 x magnification (VILIČIĆ, 2003; HORVAT, 2004). Only whole diatom valves or their representative parts are counted according to SCHRADER & GERSONDE (1978) and HORVAT (2004). Diatom measurements were made using free ImageJ software (https://imagej.nih.gov/ij/).

Determined diatoms are traditionally divided into two orders: Centrales (radial symmetry) and Pennales (bilateral symmetry).

3.3. Mineralogy

X-ray powder diffraction (XRPD) was recorded on bulk samples, insoluble rock residue and the < 2 µm fraction of samples. Some samples contained large amounts of carbonate minerals that had to be dissolved to eliminate overlapping diffraction peaks with other minerals. Carbonate fractions were dissolved by acetic acid with an ammonium acetate (1 mol dm^{-3}) buffer of pH 5 (JACKSON, 1956). Clay minerals were determined on the < 2 µm fraction of samples on oriented mounts of air dried material, and after glycol treatment, heating to 400 ºC and 550 ºC (STARKEY et al., 1984). For quantitative analyses samples with 10% internal standard (zincite) were carefully weighed and ground with about 4 ml of methanol in a McCrone mill.

For XRPD analysis a Philips vertical X-ray goniometer (type X'Pert) equipped with a Cu tube at the Croatian Geological Survey (Zagreb, Croatia) was used. Experimental conditions were: 45 kV, 40 mA, PW 3018/00 PIXcel detector, primary beam divergence 1/4 º, and continuous scan (0.02 º2Θ/s).

Quantitative analyses of the insoluble residue of samples were made using the RockJock® computer program (EBERL, 2003).

3.4. Geochemistry

For better understanding of their mineralogical composition all selected samples were also analysed by XRF methods for the major oxides and some trace elements as Ba, Cu, Ni, Pb, Sr, V_2O_5, Zn, and Zr in laboratories of the Bureau Veritas Commodities Ltd (Vancouver, Canada). A 5 g of sample was heated to determine the loss on ignition (LOI). The treated sample was then fused in a platinum–gold crucible with a lithium tetraborate. The molten material is cast in a platinum mold. Fused discs were analysed on PANalytical Axios^{mAX}.

The inductively coupled plasma atomic emission spectrometry/inductively coupled plasma emission mass spectrometry (ICP-AES / ICP-MS) methods for measuring trace elements were performed on 15 g samples. The 0.25 g split were heated in HNO_3-$HClO_4$-HF to fuming and taken to dryness. The residue was dissolved in HCl. This preparation method is only partial for some Cr and Ba minerals and oxides of Al, Fe, Hf, Mn, Sn, Ta

Table 1. Diatom distribution with their morphometrics. Legend: + scattered (< 5 %), rare (5–9 %), low abundance (10–15%), abundant (16–35%), high abundance (36–50%), dominant species (>50%) – species in fragments.

Diatom/sample	PD-1	D-01	D-02B	MA-4	JU-1	PDK	size (µm)
Centrales							
Actinocyclus octanarius EHRENBERG		+	+			+	8–35
Actinocyclus octanarius var. *tenellus* (BREB.) HENDEY		+			+	+	25–60
Actinoptychus senarius (EHRENBERG) EHRENBERG		+	+			+	20–62
Anaulus minutus GRUNOW	+					+	10–16
Anaulus simplex HAJÓS	+		+		+	+	8–12
Asteromphalus brunii PANTOCSEK		+					32–44
Coscinodiscus group *(Coscinodiscus, Hyalodiscus, Melosira, Paralia, Stephanopyxis, Thalassiosira)*	+	+	22	25	16	27	
Coscinodiscus apiculatus EHRENBERG			+				–
Coscinodiscus curvatulus GRUNOW	+	+	8	+		+	19– 37
Coscinodiscus doljensis PANTOCSEK		+	+	17	+	+	28–43
Coscinodiscus miocaenicus KRASSKE		+		+			18–21
Coscinodiscus obscurus SCHMIDT			+				38
Coscinodiscus oculus iridis EHRENBERG	+		+	+	+	+	≥40–75
Coscinodiscus perforatus var. *cellulosus* GRUNOW			+			+	–
Coscinodiscus radiatus EHRENBERG		+	+				68–72
*Coscinodiscus rothii (*EHRENBERG) GRUNOW		+	+	+			39–80
Coscinodiscus rugulosus HAJÓS		+	+				14–30
Coscinodiscus sarmaticus PANTOCSEK			+	+	+	+	12–31
Hemiaulus cf. *polymorphus*						+	46
Hyalodiscus laevis EHRENBERG						+	20–50
Hyalodiscus scoticus (KÜTZING) GRUNOW					+	+	10–40
Melosira dickiei var. *fossilis* PANTOCSEK			+				12–20
Melosira sp.					+		–
Paralia sulcata (EHRENBERG) CLEVE	+	+	+	+	+	7	12–40
Perissonoë trigona (GRUNOW) ANDREWS & STOELZEL						+	–
Pseudopodosira westii (SMITH) SHESHUKOVA-PORETZSKAYA & GLEZER			+	+		+	12–29
Rhizosolenia oligocaenica SCHRADER		+	+	+	+	+	–
Stellarima stellaris (ROPER) HASLE & SIMS	+	+	+	+	+		36
Stephanopyxis turris (GREVILLE) RALFS	+		+				63
*Thalassiosira leptopus (*GRUNOW) HASLE & FRYXELL	+	+	+	+	+	+	34–65
resting spores and siliceous cysts *(Bacteriastrum* spp., *Chaetoceros* spp, *Liradiscus* spp., *Xantiopyxis* spp., *Periptera* spp.)	6	59	+	+	6	6	–
Triceratium balearicum CLEVE & GRUNOW						+	–
T. laetum fa. *quadrata* HAJÓS						+	28
Pennales							
Achnanthes baldjiki (BRIGHTW.) GRUNOW						+	32
Achnanthes brevipes AGARDH		+					31
Achnanthes rara JURILJ		+				+	29–34
Achnanthes saeptata var. *sussedana* JURILJ					+		50
Achnanthes spp.					+		–
Amphora binodis var. *biggiba* GRUNOW	+					+	28
Amphora costata SMITH	+					+	63
Ardisonia fulgens (GREVILLE) GRUNOW						+	–
Biddulphia biddulphiana (BOYER) SMITH			+		+		54–117
Biddulphia sp.						+	–
Caloneis boryana PANTOCSEK						+	42
Climacosphenia moniligera EHRENBERG						+	16–18
Cocconeis andesitica PANTOCSEK						+	30
C. canaliculata JURILJ						+	15
Cocconeis conciata PANTOCSEK	+					+	26
C. disculus (SCHUMANN) CLEVE						+	14
C. distans GREGORY						+	40
C. evolvens JURILJ						+	23
Cocconeis fluminensis (GRUNOW) PERAGALLO	+					+	28
C. quarnerensis var. *lanceolata* JURILJ						+	20
Cocconeis cf. *sarmatica* PANTOCSEK	+					+	11–28
Cocconeis scutellum EHRENBERG	+	+	+	+	+	5	9–45
Cymatosira lorenziana var. *maior* JURILJ	+					+	34–54
Delphineis angustata (PANTOCSEK) ANDREWS		+					20–35
Delphineis surirella (EHRENBERG) ANDREWS		+	+				20
Dimeregramma angustatum HAJÓS					5		40

Table 1. continued

Diatom/sample	PD-1	D-01	D-02B	MA-4	JU-1	PDK	size (µm)
Centrales							
Dimerogramma boryanum PANTOCSEK						+	–
Dimeregramma distans GREGORY					+		39
Di. minor (GREGORY) RALFS						+	12–17
Di. minus var. *neglectum* JURILJ						+	–
Diploneis bombus EHRENBERG					+		–
Diploneis coffaeiformis (SCHMIDT) CLEVE						+	–
Diploneis crabro (EHRENBERG) EHRENBERG			+	+		+	22–45
Diploneis fusca (GREGORY) CLEVE	+					+	51
Diploneis ovalis (BRÉBISSON) CLEVE	+	+	+	+		+	34
Diploneis splendida var. *porosa* JURILJ				+			–
Diploneis subovalis CLEVE			+			+	43–57
Diploneis smithii (BRÉBISSON) CLEVE	+				+	+	30–50
Diploneis sp.		+					–
Encyonema sp.						+	–
Epithemia adnata (KÜTZING) BRÉBISSON					+		46
Epithemia zebra var. *saxonica* (KÜTZING) GRUNOW						+	–
Eunotia cf. *tenella* (GRUNOW) HUSTEDT						+	–
Fragilaria brevistriata GRUNOW	+			+		+	9–15
Fragilaria construens (EHRENBERG) GRUNOW			+			+	8
Grammatophora angulosa EHRENBERG				+		+	28–32
Grammatophora insignis GRUNOW	+				+	+	–
Grammatophora macilenta SMITH					+		25–80
Grammatophora miocaenica HAJÓS		+					28
Grammatophora robusta EHRENBERG				+			40–60
Grammatophora oceanica EHRENBERG		+	+			+	37–93
G. oceanica var. *macilenta* (SM.) GRUNOW						+	do 172
G. oceanica var. *oceanica* EHRENBERG						+	–
Grammatophora stricta EHRENBERG	+	+			+	+	22–40
G. stricta var. *biharensis* EHRENBERG						+	–
Hantzschia virgata (ROPER) GRUNOW					+		50–66
Lyrella hennedyi (W.SMITH)STICKLE & MANN	+	+				+	25–45
Mastogloia binotata (GRUNOW) CLEVE					+		40
Mastogloia lacustris (GRUNOW) GRUNOW			+		+		38
Mastogloia sarmatica JURILJ	+		+			+	69
M. splendida (GREGORY) CLEVE						+	–
Mastogloia sp.	+	+	+		9		–
Navicula latissima var. *quadrata* JURILJ				+		+	64
Navicula spp.		+			+		–
Nitzschia doljensis PANTOCSEK		+					35
Nitzschia frustulum (KÜTZING) GRUNOW			+	+			18
Nitzschia spp.	+				+		–
Opephora gemmata fa. *minor* JURILJ					+		40
Opephora marina (GREGORY) PETIT						+	17
Plagiogramma biharense PANTOCSEK			+				15
Plagiogramma bipunctatum HAJÓS	+						–
Plagiogramma staurophorum (GREGORY) HEIBERG	+		+		+	+	20–30
Planothidium quarnerensis (GRUNOW) WITKOWSKI,LANGE-BERTALOT & METZELIN					+	+	20
Rhabdonema hamuliferum KITTON					12		–
Rhaphoneis amphiceros EHRENBERG					+	+	17–32
Rh. amphiceros var. *rhombica* GRUNOW						+	–
Rh. cocconeiformis (SCHMIDT) HANNA & GRANT						+	–
Rh. nitida (GREGORY) GRUNOW						+	–
Rhaphoneis rhombica (GRUNOW) ANDREWS	+					+	34
Rhopalodia gibberula (EHRENBERG) MÜLLER	+				+	+	34
Staurosirella leptostauron (EHRENBERG) WILLIAMS & ROUND					+		10–26
Staurosirella pinnata (EHRENBERG) WILLIAMS & ROUND		+					22
Surirella subfastuosa PANTOCSEK					+		–
Synedra crystallina var. *fossilis* PANTOCSEK						+	–
Synedra fulgens (GREVILLE) W.SMITH	+		+		+		–
Synedra tabulata var. *obtusa* PANTOCSEK					+		–
Thalassionema nitzschioides (GRUNOW) GRUNOW	69	32	74	60	30	8	33–139
Thalassiotrix longissima CLEVE & GRUNOW	8		+	+	+	+	34

and Zr. Additionally, volatization during fuming may result in some loss of As, S and Sb. The analyses were done by SPECTRO CIRIOS VISION ICP-AES and Perkin Elmer Elan 6000/9000 ICP-MS. Inorganic carbon was determined by directly measuring the CO_2 gas evolved into the LECO CS230 analyzer when a prepared sample split is leached with perchloric acid.

From the last century, the Boströms' standard formula (BOSTRÖM at al., 1972) for estimating biogenic silica from geochemical data was used:

$$\text{Opal-A} = SiO_2\ (\text{total}) - 3\cdot Al_2O_3$$

We tested and abandoned this formula because of a negative result for sample MR-10B, (Tab. 5). Therefore Murdmaas' modified formula (MURDMAA et al., 1980) for extracting opal-A from hemipelagic sediments was used:

$$\text{Opal-A} = SiO_2\ (\text{total}) - 2.7\cdot Al_2O_3$$

3.5. Insecticidal efficacy

In order to test insecticidal efficacy of inert dusts against rice weevil, *S. oryzae*, 100 grams of clean soft wheat of different varieties (approx. 13% m.c.) was mixed with a determined quantity of each dust in glass jars of 200 mL. Fifty unsexed adults of *S. oryzae*, 7-21 days old were added into each jar. Inert dusts were tested in 3 or 4 different doses, depending on inert dust, and all treatments were conducted in 4 repetitions. Bioassay was kept under controlled conditions at 28±2 °C, 65 ±5 % RH and in dark. Insecticidal efficacy was estimated as lethal doses required to kill 50% and 90% of the tested population (LD_{50} and LD_{90}). The LD_{50} and LD_{90} values were calculated by Probit analysis using IBM SPSS Statistics (IBM Corp. Released, 2013).

4. RESULTS

4.1. Palaeontology

In total, in samples from Medvednica and Žumberak Mts, 111 diatom species were determined with fourteen variants and two forms. Among them, four species had not been previously discovered in Podsusedsko Dolje: *Chaetoceros didymus* EHRENBERG, *Dimerogramma minus* (GREGORY) RALFS var. *neglectum* JURILJ, *Rhaphoneis cocconeiformis* (SCHMIDT) HANNA & GRANT and *Rh. nitida* (GREGORY) GRUNOW. Approximately 90% of the diatom species are less than 50 µm in size, while larger specimens are mostly broken (GALOVIĆ et al., 2015).

Diatom distribution with their sizes is given in Table 1. to emphasise the physical character of diatomite as a potential natural pesticide. The most abundant species in the samples belong to the *Coscinodiscus* group, *Thalassionema nitzschoides* and *Chaetoceros* group with resting spores and siliceous cysts. Based on palaeontological results, the most prospective diatomite (tripolite) for commercial use would be from Medvednica Mt. (MA-4, PDK, D-02B), Fig. 3. The dominant species in sample MA-4 is

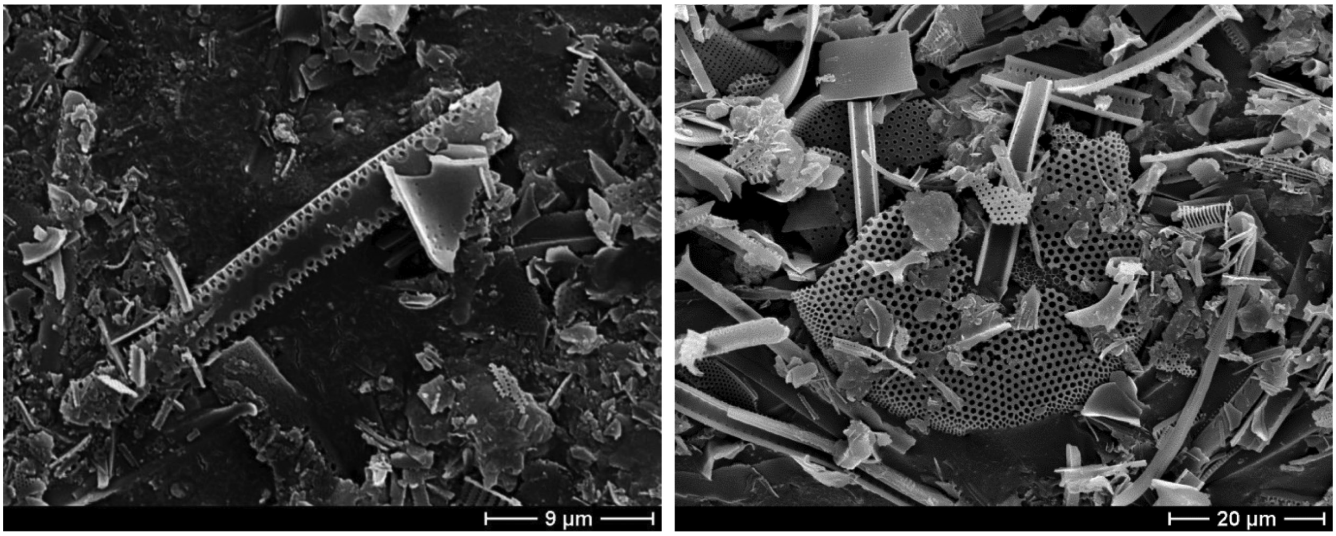

Figure 3. SEM images of partially crushed diatom *Thalassionema nitzschioides* on the left an *Coscinodiscus curvatulus* on the right from the sample D-02B.

Table 2. Quantitative mineral composition of samples (in wt. %) obtained by XRD analysis using RockJock® computer program (EBERL, 2003).
** – abundant (20–40%), * – subordinate (1–20%), + – traces (<1%)

MINERALS	MA-4	PDK	D-01	D-02B	JU-1	OP-4	OP-4A	MR-10	MR-10B
Quartz	5	3	5	4	2	2	1	2	2
Opal-A	28	12	41	50	11			3	
Opal-C		+	+			1	1		
Calcite	19	30	10	11	28	7	6	69	64
Aragonite		34			40	73	81		
Feldspar	1	2			1	1	1	1	
Pyrite				2					
Clay minerals	47	19	44	33	18	16	10	25	34
Smectite	**	*	**	**	*	*	*	*	*
Illit/muscovite	*	*	*	*	*	*	*	*	*
Chlorite	*					+			
Kaolinite		*				+	+	+	+

Table 3. Major and trace elements content measured by XRF method (in %) in Croatian diatomites and major elements in Celatom® Diatomaceous Earth Functional Additive Celatom® MN 51 standard.

Sample/ Element	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	MnO	TiO_2	P_2O_5	LOI	SUM*** of trace elements	SUM	TOT/C
MA-4	47.71*	8.81	3.85	9.63	0.92	0.37	1.43	0.07	0.41	0.10	23.44	2.218	98.96	9.65
PDK	21.26	4.51	1.98	36.29	0.67	0.47	0.59	0.03	0.25	0.11	33.33	0.002	99.51	–
D-01	55.58	6.83	2.85	5.59	1.30	0.25	1.05	0.04	0.31	0.21	22.52	2.442	98.97	8.56
D-02B	61.91	5.31	2.20	6.13	1.13	0.19	0.82	0.02	0.27	0.06	18.60	2.326	98.97	5.83
JU-1	18.65	3.15	1.34	38.10	0.49	0.21	0.44	0.04	0.16	0.10	35.22	1.048	98.95	10.07
OP-4	7.37	2.32	0.97	44.72	0.34	0.48	0.19	0.005	0.12	0.007	41.31	1.345	99.24	10.76
OP-4A	3.96	1.31	0.40	49.26	0.24	0.39	0.09	0.02	0.07	0.11	42.78	1.105	99.74	11.61
MR-10	14.21	4.73	1.57	37.15	1.89	0.24	0.83	0.07	0.24	0.08	36.53	1.541	99.08	10.42
MR-10B	16.22	5.44	2.05	36.90	1.42	0.16	0.92	0.07	0.23	0.07	35.04	0.728	99.25	9.93
Celatom® MN 51**	73.60	7.80	1.80	5.60	0.30	SUM other oxides			2.30	–	5.50	–	96.90	–

* In %
** Standard DE
*** Measured trace elements: Ba, Cu, Ni, Pb, SO_3, Sr, V_2O_5, Zn, and Zr.

Thalassionema nitzschioides in an assemblage with abundant smaller species of *Coscinodiscus curvatulus, C. doljensis* and bigger but mostly fragmented *Thalassiosira leptopus*. In the PDK dominant diatoms include *Coscinodiscus oculus iridis*, *Thalassiosira leptopus*, *Coscinodiscus stellatis* and *Paralia sulcata*.

4.2. Mineralogy

The mineralogical composition of the insoluble residues of the analysed rock samples is given in Table 2. The main components of all samples are carbonate minerals (calcite and/or aragonite), clay minerals and opaline silica (biogenic opal-A with very small amounts of mineral opal-C in some samples). Opal-A (highly disordered, near amorphous) produces a single broad peak centred at approximately 4 Å (Fig. 4) and opal-C (well-ordered α-cristobalite) at 4.04-4.06 Å (JONES & SEGNIT, 1971). All analysed samples contain quartz (SiO_2) ranging in content from 1-5%. Some samples contain smaller amounts of feldspar and pyrite.

Table 4. Trace element content in Croatian diatomites (mg/kg).

Sample/ Element	MA-4	PDK	D-01	D-02B	JU-1	OP-4	OP-4A	MR-10	MR-10B
Mo	24.4	17.4	20.2	14.4	2.2	2.3	0.8	98.7	8.0
Cu	46.8	32.9	41.5	82.9	21.3	24.6	31.0	37.9	31.4
Pb	19.4	6.9	14.7	11.8	5.6	10.8	4.3	10.4	12.9
Zn	75	33	65	63	27	23	14	48	52
Ni	113	81	89	81	31	35	13	133	62
Co	21	6	16	8	5	5	2	11	5
As	15	7	7	5	10	5	0.05	5	6
U	8	10	6	4	5	8	6	36	8
Th	8	3	6	4	2	2	1	3	3
Sr	518	3405	427	549	3438	6405	5161	1745	1653
Cd	0.8	6.2	0.8	4.5	0.8	1.1	0.3	2.9	1.6
Sb	1.8	0.9	1.3	1.7	0.6	2.2	0.4	4.1	2.0
Bi	0.5	<0.1	0.3	0.2	0.1	0.2	0.1	0.2	0.2
V	104	64	75	81	43	23	17	51	60
La	24	12	17	12	8	9	5	10	12
Ba	275	340	183	168	336	176	170	632	679
W	1.4	<0.5	1.2	0.8	0.5	0.4	0.2	0.6	0.6
Ce	54	21	35	23	15	17	9	20	24
Sn	2.4	1.0	1.6	1.3	0.7	0.8	0.6	1.1	1.3
Y	19	13	14	9	6	10	7	7	8
Nb	6	4	5	4	2	2	1	3	4
Ta	0.5	0.3	0.4	0.3	0.2	0.1	0.05	0.2	0.2
Sc	12	5	8	7	3	3	2	6	7
Li	40	23	35	29	14	12	9	25	30
Se	5	3	4	3	2	3	3	11	4
Zr	39	49	34	26	22	8	6	22	25

Celatom® Diatomaceous Earth Functional Additive Standard Celatom® MN 51:
Pb = 15 mg/kg, As = 20 mg/kg.

4.3. Geochemistry

The results of geochemical analysis for major elements are shown in Table 3. The concentration of total silica in samples varies from 3.96 to 61.91%. The measured content of SiO_2 in most samples is relatively small (< 47.71%) except for the samples from the borehole Podsusedsko Dolje (D-01 and D-02B; 55.58 % and 61.91% respectively). In comparison to the standard sample (Celatom® MN 51) the majority of analysed samples have a high content of CaO (> 35%) and relatively high content of MgO (Tab. 3). The high content of carbonate component in the samples results in high values of LOI (>18.60%).

The measured content of trace elements of nine DE samples is given in Table 4. It is significant that the concentrations of Mo and Cu are higher in samples from Medvednica Mt. than form elsewhere. The highest concentration of Pb (19.4 mg/kg), which exceed the recommended value of 15 mg/kg for diatomaceous earth of standard sample, is determined in sample MA-4 from Medvednica Mt. (Tab. 4). The samples from Medvednica Mt. and samples MR-10 and MR-10B from Banovina region also have increased concentrations of Ni (>50 mg/kg). The elevated concentrations of uranium, especially in sample MR-10 from Banovina, are connected to the higher content of organic matter.

4.4. Insecticidal efficacy on common stored pests

Results among our samples of inert dust efficacy against rice weevil (RW), *S. oryzae* are shown in Table 6., where the highest efficacy is seen in samples D-01, D-02B and MA-4.

5. DISCUSSION

The results of XRPD analyses showed that all analysed samples contain greater or lesser amounts of opal-A and quartz (Tab. 2). Quantitative analyses of the mineralogical composition of samples obtained by XRPD analyses (Tab. 2.) gave larger amounts of opal silica than results of calculations by Boströms' and Murdmaas' formulas (Tab. 5). These types of calculations are based on the assumption that the SiO_2/Al_2O_3 ratio in terrigenous clay and silty clay is almost constant, and varies from 3.5 to 2.7. According to GRIZELJ et al. (2007; 2017) XRPD analyses of Miocene pelitic sediments from the Croatian part of the PBS revealed that most of the samples contain the same mineral phases, but they are present in different quantities in different samples. The ratio of SiO_2/Al_2O_3 can vary within one clay mineral group and varies considerably within different clay mineral groups. Clay minerals have a wide range of variations in chemical composition and the variation is a composite of all the errors inherent in any

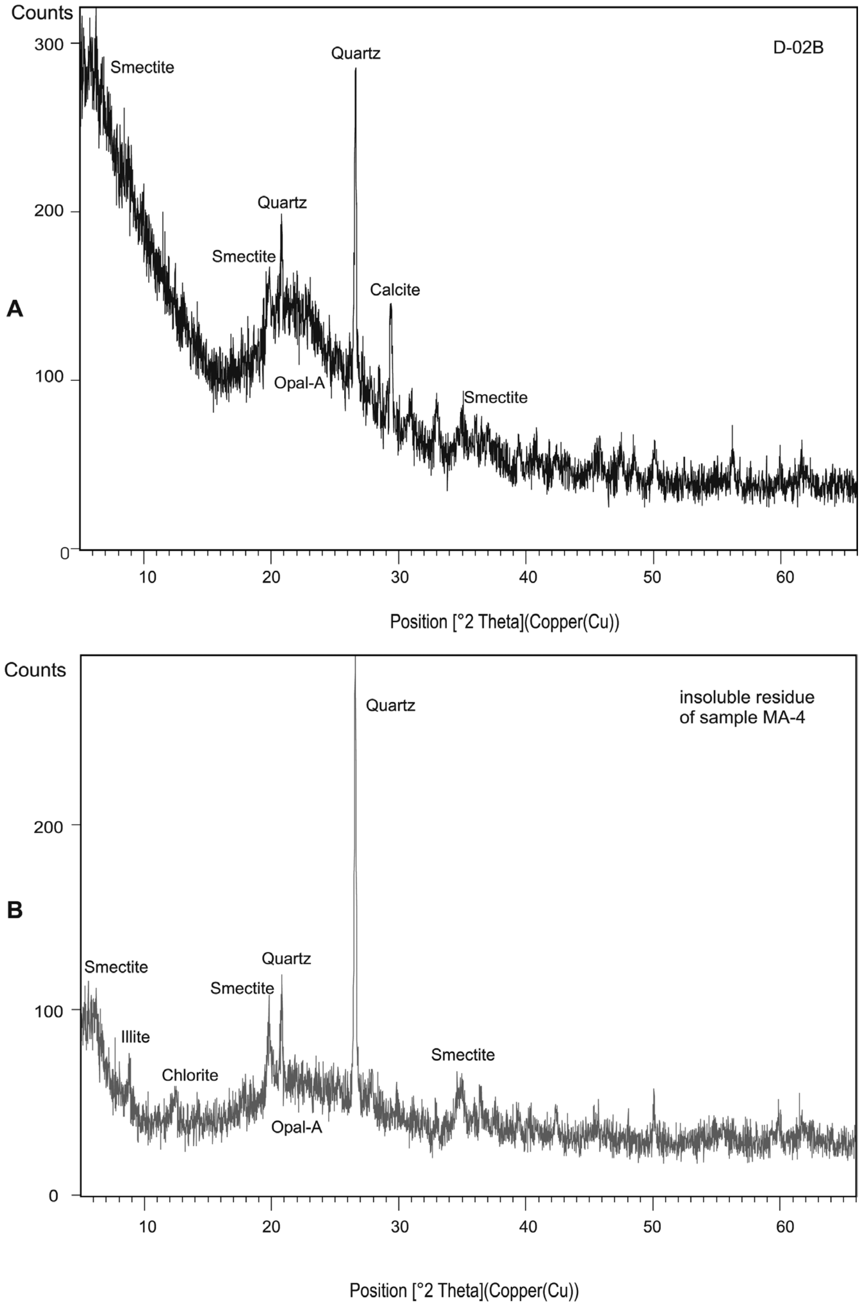

Figure 4. XRPD pattern of bulk sample D-02B, and insoluble rock residue of sample MA-4.

Table 5. Content of Opal-A^B according to standard Boströms' formula (BOSTRÖM at al., 1972) and Opal-A^M according to modified Murdmaas' formula (MURDMAA et al., 1980). SiO_2 and Al_2O_3 are from Table 3.

Element/ mineral	MA-4	PDK	D-01	D-02B	JU-1	OP-4	OP-4A	MR-10	MR-10B
SiO_2	47.71	21.26	55.58	61.91	18.65	7.37	3.96	14.21	16.22
Al_2O_3	8.81	4.51	6.83	5.31	3.15	2.32	1.31	4.73	5.44
$3{\cdot}Al_2O_3$	26.43	13.53	20.49	15.93	9.45	6.96	3.93	14.19	16.32
$2.5{\cdot}Al_2O_3$	23.79	12.18	18.44	14.34	8.50	6.26	3.54	12.77	14.69
Opal-A^B	21.28	7.73	35.09	45.98	9.20	0.41	0.03	0.02	–0.10
Opal-A^M	23.92	9.08	37.14	47.57	10.15	1.11	0.42	1.44	1.53

Table 6. Efficacy of 9 Croatian inert dusts and standard Celatom® MN 51 against *Sitophilus oryzae* (L.) adults after 7 days of exposure with LD_{50}/LD_{90} values (95% fiducial limits).

Inert dust	Lethal doses (ppm)	
	LD50*	LD90*
D-01	156.5 (62.28-213.26)	447.2 (419.88-481.90)
D-02B	300 ppm=82.5%	359.6 (240.55-422.56)
JU-1	351.4 (324.22-373.43)	606.7 (575.90-648.08)
MA-4	232.2 (183.05-266.05)	458.7 (436.21-487.52)
MR-10	693.3 (646.91-778.77)	954.1 (847.50-1159.46)
MR-10B	588.6 (564.40-623.79)	831.1 (763.36-944.09)
OP-4	421.5 (346.71-460.50)	867.7 (750.76-1161.96)
OP-4A	534.8 (517.44-555.17)	744.4 (699.14-813.40)
PD-1	362.8 (314.80-391.50)	726.4 (668.39-816.94)
Celatom® MN 51	300 ppm=83.5%	334.1 (86.64-399.00)

*LC50 and LD90 expressed as parts per million (ppm), Confidence limits (CL) are given in parentheses

analytic analysis: operator, instrument, method, beneficiation, sampling (WEAWER and POLLARD, 1973; WEAVER 1989).

According to the geochemical analyses there is a significant variation in the total silica (SiO_2) content in samples collected on the Medvednica Mt. (21.26% sample PDK, 47.71%, sample MA-4, and 61.91% sample D-02B) (Tab. 3). This is particularly evident at the Podsusedsko Dolje site, where the distance from the sample PDK and samples D-01 and D-02B is about a kilometre. Laterally marked variations in the silica content of the investigated diatomites are most likely the result of the marine geomorphology of the depth and its variations during the mid-Sarmatian, as well as diagenesis and tectonic processes that modify the composition and crystalline phase of the mineral. This also applies to the SiO_2 content at the Jurjevčani (18.65%) site (Žumberak Mt.), which is significantly lower than the average silica content on the Medvednica Mt. (mean = 48.63%) considering that the direct distance from the Podsusedsko Dolje site is only 25 km today. This is in accordance with their origin where the most prospective diatomites from Medvednica Mt. belong to the upwelling zone of the temperate climate with seasonal blooming (GALOVIĆ & BAJRAKTAREVIĆ, 2006), while that from Žumberak belongs to a more coastal marine area. The concentration of silica in the samples from Banovina is lower than that registered in the samples from the Medvednica and Žumberak Mts. (Tab. 3). The lowest content of SiO_2 is found in the samples from Slavonia (OP: ≤7.37%) which is why we advocate tripolite instead of ordinary diatomite. The SiO_2 concentrations in those analysed samples are more depleted in comparison with the standard Celatom® MN-51. The concentration ranges from 3.96 to 7.37% SiO_2 (Tab. 3). The insecticidal effect of DEs against stored product insects greatly depends on the amount of biogenic silica (KORUNIĆ, 2013). Samples with the lowest concentration of silica have the highest carbonate component (CaO) content. According to FIELDS & MUIR (1995) calcium oxide also has some insecticidal activity. Samples JU, OP and MR have enhanced CaO (36.90-49.26%) which could be a reason why they showed promising insecticidal efficacy in preliminary testing. All samples except D-01 and D-02B have much higher contents of CaO compared to the standard sample (Tab. 3). The MgO content is in most samples associated with the CaO content which is probably due to the originally precipitated dolomite in association with calcite. The content of alumina depends on the amount of the clay component in the sample. In relation to Celatom® MN 51 only one analysed sample, MA-4, has a slightly elevated content of alumina (Al_2O_3), which is probably a part of the detected smectite which increased its adsorption (Tab. 2 and 3). The Fe_2O_3 content is slightly higher in all analysed samples from the Medvednica Mt. as well as in the sample MR-10B (Banovina) in comparison to Celatom® MN 51. The highest concentration of iron registered in the sample MA-4 from the Medvednica Mt. probably originates from the chlorite mineral group occurring in the sample (Tab. 2). The sum of all other oxides (Na_2O, K_2O + MnO + TiO_2 + P_2O_5) in the analysed samples of Croatian diatomites is smaller than their content in Celatom® MN 51 (Tab. 3). Among the trace elements, only the concentrations of potentially toxic lead (Pb) and arsenic are of importance. The amount of Pb exceeds the limit values relative to Celatom® MN 51 (15 mg/kg) solely in the sample MA-4 (19.4 mg/kg) from Medvednica Mt., while the concentrations in all other samples are lower. Enhanced lead might be a result of the weathering of the galenite ore deposit there. The content of arsenic (As) is significantly lower in all analysed samples compared to sample Celatom® MN 51 (Tab. 4).

The highest efficacy against the rice weevil, *S. oryzae* showed in samples D-01, D-02B and MA-4 (Tab. 6). Samples D-01 and D-02B have smaller amounts of quartz (4-5 %) and maximum amount of opal-A (41-50%), as BSi, which make them suitable for commercial usage as DE (Tab. 2, Fig. 4). As a BSi component, *Thalassionema nitzschioides* dominate in the assemblage with abundant, bigger species (65-80 μm) of *Coscinodiscus* group (*Coscinodiscus oculus iridis*, *Thalassiosira leptopus*, *Coscinodiscus radiatus*, *Coscinodiscus stellatis*, *Coscinodiscus rothii*, *Coscinodiscus perforatus* var. *cellulosus*), which are mostly fragmented, and with their smaller representatives (*Coscinodiscus curvatulus, Coscinodiscus doljensis, Coscinodiscus sarmaticus*, *Coscinodiscus rugulosus*, *Paralia sulcata*), giving better efficacy of D-02B sample as DE like MA-4 (Fig. 3, Tab. 1). The relatively high content of opal-A (28%) and a small amount of quartz (5%) characterised sample MA-4 (Tab. 2). In previous testing (LIŠKA et al., 2015) of samples (MA-4, MR-10, MR-10B, OP-4, OP-4A and PD-1) against *Tribolium castaneum* (HERBST) and *Rhyzopertha dominica* (FAB.) the highest efficacy (7 and 14 days after treatment) showed in sample MA-4, reaching *T. castaneum* mortality of 61.50% and 99%, respectively, and 25.50% and 34.0%, respectively against *R. dominica*. In addition, there were no statistical differences of mortality rates with the standard Celatom® MN 51. According to ZIAEE et al. (2013) each diatom species could have its own mode of physical action, regarding the number and size of pores and distribution of striae and if it is greater, the insecticidal activity would be higher. The results on diatom morphometrics show that the most efficient diatomites consist of mid-sized (<45 μm) *Coscinodiscus* (*Coscinodiscus curvatulus* and *C. doljensis*) with numerous smaller pores, and lancet shaped *Thalassionema nitzschioides* that make up its active surface (Fig. 3). This is in agreement with KORUNIĆ (1998), that diatoms with discoid, flattened and linear body shapes better cover insect cuticle in relation to diatoms of cylindrical and round shapes. DE particles can effectively adsorb epicuticular lipids from the cuticle surface of the treated insects (ROHITHA PRASANTHA et al., 2015) and these phenomena may have a great effect on efficacy against insects. According to KORUNIĆ et al. (2016), larger available active surfaces have a higher sorption capacity for the lipids and are more effective desiccants. In the experiment conducted by KORUNIĆ & FIELDS (2016) silica aerogel Sipernat 50S with oil sorption of 3.23 ml/g dust was significantly more effective against *Sitophilus granarius* (LINNAEUS) in comparison with other DEs with oil sorption from 1.13 to 1.96 ml/g dust. Inert dusts MA-4 with oil sorption of 1.23 and Celatom® MN 51 with oil sorption of 1.20 were equally effective against rice weevil *Sitophilus oryzae* (LINNAEUS) and granary weevil, *S. granarius*. Nevertheless, noticeable sponge spicules and silicoflagellates in samples may damage the digestive tract or breathing organ, also resulting in internal desiccation or suffocation of the insects (JACKSON & WEBLEY, 1994), which increases efficacy as well. Sample MR-10 from Banovina region has only 3 % opal-A, while sample MR-10B did not contain opaline silica. Two samples from Slavonia OP-4 and OP-4A contain small amounts of opal-C (Tab. 2). Based on the palaeontological results, the sporadic appearance of sponge spicules was noticed only in samples of OP, which is in agreement with the results of Murdmaas' modified formula (Tab. 5). These samples are partially modified by calcinations in the process of diagenesis. It is well known that during diagenesis siliceous deposits undergo mineralogical changes from non-crystalline biogenic silica (opal-A) to microcrystalline opal (opal-CT/C) to microcrystalline quartz (SiO_2) (WILLIAMS et al., 1985; FLÖRKE et al., 1991; CADY et al., 1996; LYNNE & CAMPBELL, 2004). Micro-crystalline silica minerals (quartz) have been shown to be carcinogenic if inhaled. In addition, crystalline silica is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC, 1997). DEs registered as insecticides generally have less than 6% of quartz, like all analysed samples. However, the use of a proper dust mask, or the use of low crystalline silica DE can protect against this health risk (DESMARCHELIER & ALLEN, 2000). Very small amounts of opal-C were contained in samples PDK and D-01 (Tab. 2). Sample PDK appeared to have the best outcome on RW with inert dust less than 25 microns even after 5 days. This is in agreement with the fact that smaller particles of DEs (<45 µm) are significantly more effective than larger particles (KORUNIĆ et al., 2011). Species are mostly smaller than usual, but this is in accordance with their origin in the upwelling zone and with seasonal changes during the Sarmatian, that limited their rapid growth in the blooming period (JURILJ, 1957; GALOVIĆ & BAJRAKTAREVIĆ, 2006). Nevertheless, sample PDK has a high content of carbonate minerals (calcite and aragonite), and a smaller amount of opal-A (12%), and quartz (3%) (Tab. 2). Despite lower concentrations of amorphous silica in samples MA-4 (28%) and PDK (12%), in combination with the increased content of smectite gives even better absorption properties. Clays are also known as inert dusts like DEs (MACELJSKI & KORUNIĆ, 1972; BANKS & FIELDS, 1995; SUBRAMANYAM & ROESLI, 2000) that also influenced better adsorptions here. In almost all the analysed samples smectite is the dominant clay mineral. It has a 2:1 layer structure, with a large base exchange capacity (60–130 meq/100 g) and will readily adsorb cations such as H^+, Na^+, Ca^{2+}, and Mg^{2+} (WEAVER, 1989). Interlayer cations are hydrated and replaceable, resulting in the swelling and dehydration characteristic for smectite (SLOVENEC & BERMANEC, 2003).

6. CONCLUSION

Sarmatian diatomites from Medvednica Mt. have different physical properties and efficacy on grain store insects. The analysed Croatian diatomites have significantly lower concentration of silica in relation to the standard DE. Diatomites from borehole (D-01, D-02) have the highest amorphous silica component (41-50%) and small amounts of quartz (4-5%) which makes them suitable for commercial usage as DE. They exhibit the highest efficacy on stored products insect rice weevil *S. oryzae*, together with inert dust from Medvednica Mt. (MA-4, PDK). Based on palaeontological results the most efficient analysed DE consists of mid-sized planktonic *Coscinodiscus* group of species where *Thalassionema nitzschioides* dominate which is positively correlated with their absorption. Even much smaller concentrations of amorphous silica in sample MA-4 (28%) and PDK (12%), but in combination with increased content of smectite gives even slightly better absorption property. The higher level of CaO (9.63-36.29) in correlation with Celatom® MN 51 (5.60) probably also increased efficacy. Ca (II) ions are known to improve flow rate in modified diatomite (BAZHAL et al., 1975). Probably because of all mentioned parameters diatomite MA-4 and smaller fraction of PDK show no differences of mortality rates with the standard Celatom® MN 51. Unfortunately, the small thicknesses of all prospective Croatian diatomites (approx. 48 cm) and the slightly higher content of lead in MA-4 sample make them inadequate for classical usage as DE (MA-4= 19.4. mg/kg, Tab. 4). According to the standard sample Celatom® MN 51 the allowed maximum Pb concentration is 15 mg/kg. The content of quartz in the samples is 1-5 %. Concerning insecticidal effects against the rice weevil, *S. oryzae* and taking into account the small thicknesses of all prospective Croatian diatomite layers, there is the possibility of mixing these inert dusts with some other natural compounds, with other insecticide effects, to provide the same (or even higher) insecticidal level.

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