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ORIGINAL ARTICLE

RP-HPLC and transcript profile indicate increased leaf caffeine in *Coffea canephora* plants by light

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ABSTRACT

Light is a survival quotient for all photosynthetic plants and its reception is very complex due to direct regulation by photoreceptors and their downstream transcriptional factors or indirectly by circadian rhythm. Shade-grown coffee cultivation though less productive than the sun tolerant varieties, pose high potential as benefit to the environment. Other than high nutrient soil associated with shade-cultivated coffee, light is another important difference when compared to full sun cultivation practice. It is thus important to study if light has a role in accumulation of caffeine - the most undesired compound in coffee. Light irradiation of suspension cultures of *Coffea arabica* enhances caffeine content. However, no such study is available on whole plants, which are anticipated to act in accord with organismal homeostasis. Moreover, the promoter of theobromine synthase-like gene involved in caffeine biosynthesis carries several light responsive motifs. In this report, it is shown that in complete darkness the caffeine content in young leaves of 1 year old seedlings is very low (0.094±0.003 mg/100 mg tissue dw.). However, it increases to 5.9 folds within 6hrs of exposure to light. In addition, caffeine content drops (0.218±0.03 mg/100 mg tissue dw.) when light exposed plants are returned to complete dark. Transcript analysis further reveals that this difference is due to regulation of the caffeine biosynthetic genes. A further discussion to the effect of dark and light on levels of caffeine is also provided. Though cup quality of shade-grown coffee is indefinite, this study clearly demonstrates the role of light in regulation of caffeine biosynthesis.

Key words: Coffea; N-methyltransferase; Light; Transcript profiling; High Performance Liquid Chromatography.

INTRODUCTION

Plants being sessile organism have higher constraints to evolve complex sensing and signaling mechanisms to monitor even small environmental changes [1]. The adequate response to various environmental changes like light, temperature, humidity variation or nutrient and water deprivation as well as responses to biotic and abiotic stress plays a central role in survival of the plant species. One of the major outcomes of such environmental variation is exhibited through a cascade of cellular process leading to changes in transcript profiles of responsive genes and finally culminates through the action of effector molecules. Light is an important subsistence factor for photosynthetic plants. Light regulation, more than being complex, is far from understood. Photoreceptors (e.g. phytochromes) and circadian rhythm are involved in the direct and indirect response to

light, respectively [2]. Signal transduction through photoreceptors relies on proteins like GT-1 and GATA as the major transcription factors [3].

Coffee is a native of the African continent where the wild species inhabit the under-storey of the tropical forests [4]. Today a major proportion of world produce is cultivated in Brazil (around one third of the total world produce) (www.ico.org). Coffee cultivation practice around the globe is shifting with the use of sun tolerant varieties that drastically improve the yield [5]. However, they come with serious failings like deforestation, damage to biodiversity, high inputs of pesticide and fertilizers and soil/water deterioration [6]. India which is the sixth largest coffee producer (311.52 thousand tones in the year 2013-2014) mainly relies on the traditional shade grown coffee and is one of the countries know to grow coffee in the most eco-friendly conditions [7]. Moreover, apart from being branded as premium coffee, shade grown coffee cultivators have advantage of hidden income in the form of intercropped plantations like pepper, cardamon, orange, banana and vanilla [8]. Shade has also been known to improve cup quality of coffee by increasing reducing sugars and decreasing chlorogenic acid content in fruits [9, 10]. As the debate on benefits of shade grown coffee continues and with recent reports concluding no significant reduction in productivity of some shade grown Arabica plantations, one point is clear that the benefits of shade-grown coffee plantations could be possibly expanded by careful design of shade canopy and the genotype of plantations used [11, 12]. Thus, it is an opportunity to study the effect of light on the biosynthesis of caffeine-the major molecule of concern with respect to over consumption of coffee [13].

Caffeine metabolic pathway is an extension of nucleotide metabolism through the more ubiquitously found compound xanthosine. The biochemical pathway is well characterized and the genes (*N*-methyltransferases) involved in the biosynthesis have been cloned and sequenced from coffee, tea, cocoa and guarana, where it occurs sporadically through convergent evolution [14, 15]. Xanthosine is sequentially methylated at the N_7, N_3, and N_1 positions of the purine ring forming 7-methylxanthosine, theobromine and ultimately caffeine [reviewed by 16]. The *N*-methyltransferase genes are characterized as xanthosine methyltransferase (XMTs), 7-methylxanthine methyltransferase or theobromine synthase (MXMT) and 7,3-dimethylxanthine methyltransferase or caffeine synthase (DXMT). Studies on *C. arabica* NMTs reveal that two variants of theobromine synthase are present, one coding for 378 amino acids (*CaMXMT1*, CTS1) and the other for 384 amino acids (*CaMXMT2*, *CTS2*) [reviewed by 17]. With the completion of coffee genome sequence, it is been shown that evolution of caffeine biosynthesis in coffee is distinct from that in tea and cocoa and it has occurred at a faster rate in coffee lineage due to highly duplicated NMT gene family comprising nineteen member genes in *C. canephora* [18]. The promoter of theobromine synthase (the 378 amino acid variant) gene from *Coffea canephora* indicate the presence of numerous light responsive motifs like GT-1 box and GATA box, most of which appear to be located in clusters and implicate a role of light in regulation of caffeine biosynthesis [19]. Earlier results of tissue culture explants also indicate higher caffeine accumulation in response to light [20]. However, the effect of light on accumulation of caffeine has not been precisely addressed at the whole plant/seedling level. This study is thus proposed to investigate the effect of light and complete dark conditions on caffeine accumulation in *Coffea canephora* var. robusta cv. S 274, both at biochemical and molecular levels, in young leaves of seedling under controlled laboratory conditions. S 274 was developed at the Central Coffee Research Institute, India, through mass selection, as one of the few released Robusta cultivars in the country. The new seedlings are grown from seed stocks of the mother plantations [21].

2. MATERIALS AND METHODS

2.1. Plant materials and treatments

One year old *C. canephora* var. robusta S274 seedlings maintained in soil pots in green house in complete dome and a 30 sec: 30 sec sprinkler irrigation were soaked in water and washed in slow running water to remove soil adhering to the roots with minimal injury to plants. The seedlings were acclimatized to 400 ml liquid 1x Hoagland's nutrient medium for three days in green house and then subjected to complete dark conditions for

48hrs, 24±2^{0}C in dark chambers in tissue culture room. Just prior to altering the circadian cycle of the seedlings by placing them in dark for 48hrs, young leaves were sampled and labeled as 'Control' (at 7:00hrs IST). Sampling was repeated after the 48hr dark period and labeled as 'Dark' (7:00hrs, IST). The seedlings in dark were then exposed to white fluorescent (400 Lux) for 6hrs (13:00hrs IST) and 12hrs (19:00hrs IST) for the sampling of leaves as labeled 'Light-6hrs' and 'Light12-hrs', respectively. As another control, the light exposed plantlets were placed back in dark for the next 48hrs ('Dark-retained'; 19:00hrs IST). The entire experiment was carried out at controlled laboratory conditions with minimal variables except for the light conditions. First pair of leaves from the apex was extracted for biochemical estimation of the methylxanthines (7-methylxanthine, theobromine, caffeine and theophylline) and isolation of total RNA.

2.2. Methylxanthine estimation

The leaf sample (500 mg) was dried at 37^{0}C overnight and ground to fine powder using mortar and pestle. Methylxanthines were extracted by boiling in 1 ml of 50% methanol:water for 10 minutes at 80^{0}C. The extract was centrifuged at 11000 g for 15 minutes and the pellet was re-extracted similarly four times. The extracts were pooled, freeze-dried for 16hrs and dissolved in 5 ml of 50% methanol:water. Methylxanthines were estimated in C-18 Bondapack column in RP-HPLC using 0-40% gradient of methanol in 50 mM sodium acetate pH 5.0 [22]. Parameters were controlled by Shimadzu LC10 liquid chromatography equipped with a dual pump and a UV spectrophotometer (model SPD 10A) (270 nM). All analysis was carried out in triplicates and the entire experiment was repeated. A total of three plants were used for each measurement since much variation was not observed in treatments carried out in controlled conditions.

2.3. Statistical analysis

The quantification of methylxanthines was carried out based on regression plot of premixed standards containing known quantities of the methylxanthines (7-methylxanthine, theobromine, caffeine and theophylline). Peaks obtained in the chromatographs of samples were verified by spiking each sample with the standard premix. One way Annova with Duncan's multiple range test (DMRT) and Dunnet's test criteria was used to authenticate statistical significance of the differences in mean concentration of methylxanthines. Validation of HPLC results was carried out with the four-concentration standard premix according to method described elsewhere [23].

2.4. Transcript profiling

Total RNA was isolated using Nucleospin II RNA isolation kit (Machery-Nagel, GmBH and Co., Düren) with on-column DNase treatment. Minus-reverse transcriptase-PCR was used prior to semi-quantitative analysis for confirming absence of genomic DNA contamination in the preparation. RNA (1 µgm) was primed with oligo-dT_{18} (Qiagen) and reverse transcribed using ImPromII reverse transcriptase (Promega). 1:5 diluted first strand cDNA was used as a template in a 10 µl reaction volume with 400 nM of each forward and reverse primers, 200 µM dNTP and 0.5 units of *Taq* DNA polymerase (Sigma-Aldrich). Primers (Table 1) designed for the first (XMT), second (MXMT) and third NMT (DXMT) were used to study the transcript levels of the three genes involved in the core caffeine biosynthetic pathway. Apart from this, primers specific to the 378 amino acid variant of MXMT (MXMT1) was also included in the analysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Ubiquitin (UBI) were used as reference genes. The cycling conditions include initial denaturation at 94^{0}C, 2 minutes, followed by 40 cycles of denaturation at 94^{0}C, 20 seconds, annealing at 55^{0}C, 30 seconds and extension at 72^{0}C, 20 seconds. The entire PCR product was resolved on a 2% agarose gel and visualized by ethidium bromide staining.

3. RESULTS AND DISCUSSION

3.1. RP-HPLC validation

High accuracy of quantification was obtained using four-concentration standard premix (101.68 ±4.11 for 7-methylxanthine (7-MX), 101.67±3.56 for theobromine (Tb), 99.49±4.68 for caffeine (Cf) and 102.4±6.32 for theophylline (Tp). Coefficient of Variation (CV) was estimated to be 3.14% for

7-MX, 1.72% for Tb, 2.74% for Cf and 3.68% for Tp. The detection and quantification limit was very low and was found to be suitable for estimation of methylxanthines in the samples. Limit of Detection (LOD) ranged from 0.0616 µg for Tp to 0.0624 µg for 7-MX to 0.0702 µg for Tb to 1.8254 µg for Cf whereas, Limit of Quantification (LOQ) was 0.1866 µg, 0.1889 µg, 0.2128 µg and 5.5289 µg, respectively. The regression coefficient was greater than 0.9 (R^2=0.91 for 7-MX, R^2=0.995 for Tb, R^2=0.994 for Cf and R^2=0.999 for Tp).

Table 1. List of NMT primers used in the study along with their sequence and the accessions used for primer designing.

Gene	Primer name	Sequence	Accession numbers of sequences used for primer designing
Xanthosine methyltransferase (XMT)	NMT123-F NMT1-1R	5' TGTAAAGGAGTTGAATTAGACGCC 3' 5' CTGCTTTAATATGTTCATCGTCAAT 3'	AB048793 (CaXMT1), AB084127 (CaXMT2), DQ422954 (CcXMT1), AB034699 (CmXRS1).
Theobromine synthase (MXMT/ MXMT1)	TSRT-1F NMT2-1R	5' ATAGTTTCAATATTCCATTCTTTAC 3' 5' GGGTTCGTAAACTGATCTAATTAAT 3'	AB048794 (CaMXMT1), AB084126 (CaMXMT2), AB034700 (CTS1), AB054841 (CTS2), DQ348077 (PG-1), DQ348078 (PG-4), DQ010011 (PG-5).
	MXMT1-1F MXMT1-1R	5' ACCCAGTAAGATCCCATGAACA 3' 5' GAGAGAAATGATAAGATTATTATAGC 3'	AB048794 (CaMXMT1), AB034700 (CTS1), DQ348077 (PG-1), DQ348078 (PG-4), DQ010011 (PG-5).
Caffeine synthase (DXMT)	CcTS3x-3F NMT3-1R	5' ACGTGGCCGAATGCTCCTTAC 3' 5' GGTTCGAAAATTGATCTAACGACA 3'	AB086414 (CaCCS1), AB084125 (CaDXMT1), DQ422955 (CcDXMT1).
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	GAPDH-F GAPDH-R	5′ ACGATAGGTTTGGCATTGT 3′ 5′ GTGCTACTGGGAATAATGTT 3′	GQ372995
Ubiquitin (UBI)	Ubi-F Ubi-R	5′ GGGTGGAGGAGAAAGAAGGAAT 3′ 5′ CTCCACCTCTCAGAGCAAGAAC 3′	AF297089

3.2. Methylxanthine estimation

Caffeine content was found to be strongly regulated by light as observed by the comparison of caffeine content in leaves from light (Light-6hrs) exposed seedlings and in leaves extracted from seedlings kept in dark conditions (Figure 1). The caffeine content in young leaf of Hoagland's medium acclimatized plant whose circadian cycle was not disturbed (Control) was not very high (0.399±0.002 mg/100 mg tissue dw.). This may be related to cultivar specific trait or possibly due to hydroponic medium. [24], in their analysis of control versus siRNA knockdown *C. canephora* var. Conilon lines, for example, report an average of 0.844 mg/100 mg tissue dw. caffeine in 1 year old control seedlings under normal photoperiod. Additionally, it was noticed in our experiments that caffeine levels dropped drastically after the circadian undisturbed seedlings were kept in dark for 48hrs (0.24 fold; 0.938±0.003 mg/100 mg tissue dw.) (Dark). When seedlings kept in dark were exposed to light there was a 5.9 fold increase (0.554±0.031 mg/100 mg tissue dw.) in caffeine within 6hrs (Light-6hrs) exposure and came to levels of control plants within 12hrs (0.417±0.018 mg/100 mg tissue dw.) (Light-12hrs).

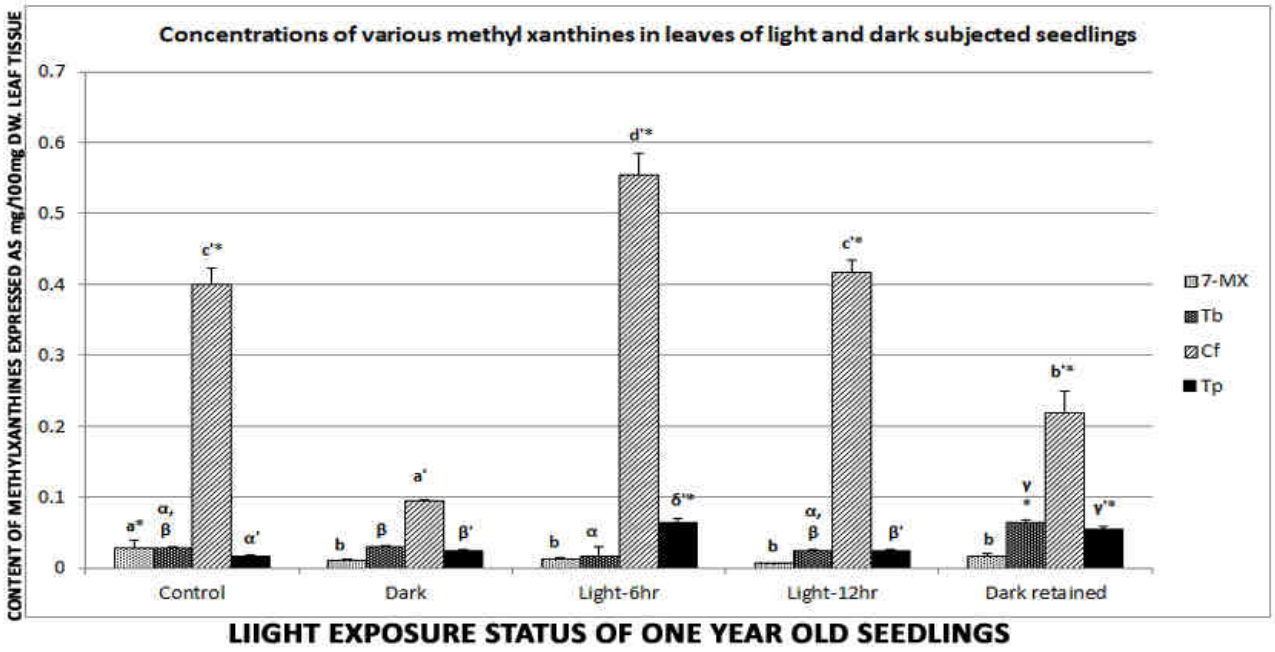

Figure 1. Contents of methylxanthines in young leaves of *Coffea canephora* seedlings on light exposure.

Percentage of 7-methylxanthine (7-MX), Theobromine (Tb), Caffeine (Cf) and Theophylline (Tp), as expressed in mg/100 mg (dw.), in the first leaf from apex of seedlings under different light conditions. The samples include normal day: night seedlings (Control or circadian un-disturbed plants), which was sampled after acclimatizing in liquid Hoagland's medium and just prior to Dark exposure, seedlings placed in controlled tissue culture room in complete darkness for 48hrs (Dark), seedlings from the dark treatments exposed to 400lux white light for 6hrs (Light-6hrs) and 12hrs (Light-12hrs) and seedlings placed to complete darkness post the 12hr light treatment (Dark-retained). Alphabets (a, b; α, β, γ; a', b', c', d'; α', β', γ', δ') indicate the mean group of the four methylxanthines in study as estimated by Duncan's multiple range test. * indicates significant mean difference in Dunnett's test in comparison to samples from seedlings in dark at a significance of $P<0.5$.

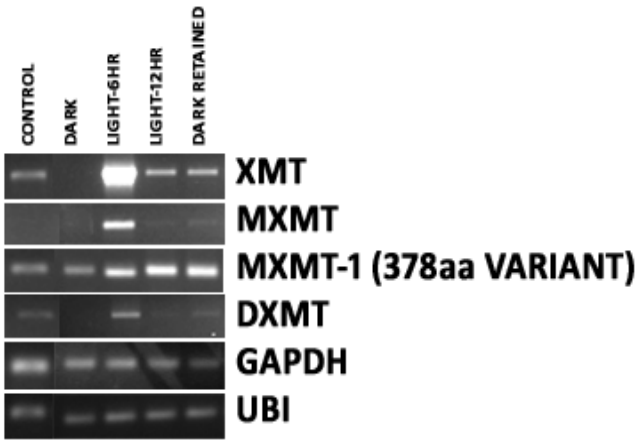

Figure 2. Transcript profiling of caffeine biosynthetic NMT genes in young leaves of *Coffea canephora* seedlings on light exposure.

Semi-quantitative RT-PCR of leaf RNA for the first NMT (XMT), second NMT (MXMT) and the third NMT (DXMT) genes of the pathway in response to light exposure as in Figure 1. The amplicons specific to the 378 amino acid variant of *CaMXMT1* (MXMT1) is also depicted. Glyceradehyde 3-phosphate dehydrogenase (GAPDH) and Ubiquitin (UBI) was used as reference genes. The samples are similar to the ones mentioned in Figure 1.

This may be indicative that the outcome of circadian rhythm of 12hrs: 12hrs (day: night) cycle on caffeine biosynthesis, if any, was restored within 12hrs of light exposure (Light-12hrs) or that it may not be very significant in the regulation of caffeine metabolism. As studied earlier, caffeine biosynthetic activity was restored in cell suspension cultures in a lag of one day after exposure to light, though *de novo* synthesis through purines required 6 days and maximal caffeine accumulated on the tenth day [20]. However, our study was intended to study the response of whole seedling to light and hence was not carried out in long duration. It was also noticed in our study that the caffeine levels decreased when the 12hrs light-exposed plants were placed back in dark (Dark-retained; 0.218±0.031 mg/100 mg tissue dw.), though not to the extent of Dark samples (Figure 1). Other methylxanthines like the precursors, 7-methylxanthine and theobromine as well as the major degradation product theophylline did not show any interesting pattern except that 7-methylxanthine content was

always lower in leaves of plants in dark and dark followed by light exposure with statistical significance at P<0.5 when compared to control plants and not to dark treated plants (Table 2). Further studies may be required to demonstrate the role of day: night cycle in the regulatory aspect of caffeine biosynthesis.

3.3. Transcript profiling of caffeine biosynthetic NMTs

The expression of the three *N*-methyltransferases reduced when the control plants were placed in complete darkness thus leading to drastic reduction in caffeine content as mentioned earlier. It is interesting that though the 378 amino acid variant of MXMT (MXMT-1) is responsive to light, exhibiting many fold increase, the basal level is still expressed even in dark comparable to leaves from control plants. The XMT, MXMT and DXMT transcripts accumulate several fold higher reaching peak levels within 6hrs of light exposure before falling back to amount comparable to leaves of the control plants by the 12th hour of light exposure (Figure 2). NMT levels were low in leaves of plants from Dark-retained condition except the MXMT-1 variant. Theobromine concentration remains constant. However, due to no reduction in the levels of MXMT1 like transcript in 'Dark-retained' plants, the leaves may contain higher theobromine content (2.7 fold higher than 12hrs light exposed samples (Figure 1).

Table 2. Statistical analysis of mean methylxanthine content in leaves of experimental and control (undisturbed circadian rhythm) plants.

Methylxanthine	Comparison	Mean difference Dunnett's (2-sided)
7-methylxanthine	Dark vs. Control	-0.016474435*
	Light 6hrs vs. Control	-0.015081601*
	Light 12hrs vs. Control	-0.021505022*
	Dark retained vs. Control	-0.010881166
Theobromine	Dark vs. Control	0.003237439
	Light 6hrs vs. Control	-0.009845597
	Light 12hrs vs. Control	-0.002852580
	Dark retained vs. Control	0.037636195*
Caffeine	Dark vs. Control	-0.305622403*
	Light 6hrs vs. Control	0.154547485*
	Light 12hrs vs. Control	0.017668903
	Dark retained vs. Control	-0.181434985*
Theophylline	Dark vs. Control	0.006976648
	Light 6hrs vs. Control	0.046841060*
	Light 12hrs vs. Control	0.007988875
	Dark retained vs. Control	0.038931112*

* indicates significance at P<0.5 in the comparison of means between experimental samples vs. control in Dunnett's 2-tailed test criteria of one way Anova analysis.

Although this study clearly indicates the importance of light for caffeine accumulation in *C. canephora* seedlings in a light-dark experimental setup, the effect of intensity of light on caffeine content is still uncertain and neither do we have much support from other studies carried out with green-house plants [25]. Furthermore, mate and tea showed decrease or no change in caffeine content,

in high light conditions [26-28]. A study on optimized field grown *C. arabica* plants indicate that shade (45% sunlight), though improved the general cup quality of coffee, showed slight increase in bean caffeine content [29]. Other environmental factors like high-altitude show significant reduction in caffeine and other metabolite content of mature berries [30, 31]. Caffeine turnover is complex and is known to vary between species [16, 32]. Furthermore, a recent study concludes action of different transcriptional and evolutionary mechanism of regulation of caffeine in Arabica and Robusta [33]. It may be thus concluded that regulation of caffeine under shade and full sun may be very complex and may need analysis of other assumptions of caffeine metabolic pathway too. Further data on, for example, turnover of xanthine derivatives using real-time field experiments determining the dynamics of degradation of the caffeine and upstream xanthine derivatives would provide the mechanism of this phenomenon. However, this *in vitro* study clearly indicates that light has a major control in expression of NMT transcripts and is responsible for normal or over accumulation of caffeine in young leaves of coffee seedlings.

AUTHORS' CONTRIBUTION

Conception and design: PG, AK and SPS; Development of methodology: AK, SPS and PG; Acquisition of data: GK, SPS and AK; Analysis and interpretation of data, writing, review and/or revision of the manuscript, administrative, technical or material support: AK and PG; Study supervision: PG. All authors are involved in drafting the manuscript, read and approved the final manuscript.

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TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Comparison between the toxicity of plant origin and synthetic pesticide against fresh water fish *Cirrhinus mrigala*

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ABSTRACT

This study was done to check the intensity and mode of action of plant origin and synthetic pesticide, which is generally, apply as piscicide for cleaning the fish culture pond. In this study plant, origin piscicide Apigenin extracted from leaves of *Jatropha gossypifolia*, and Rutin from leaves of *Codiaeum variegatum* plants of family Euphorbiaceae and synthetic pyrethroid pesticide (Cyperstar) purchased from market. The LC_{50} values of Apigenin (83.26, 68.56, 62.11 and 44.92 mg/L), Rutin (58.43, 52.32, 44.40 and 43.62 mg/L) and Cyperstar (0.009, 0.007, 0.006 and 0.005 mg/L) at exposure of 24h, 48h, 72h or 96h, respectively. It is also evident from this study; all these plant origin and synthetic pesticide significantly inhibit the activities of enzyme acetylcholinesterase (AChE), acid and alkaline phosphatase. The anti-enzymatic activity as well as the mortality caused by the Apigenin, Rutin and Cyperstar were time as well as dose dependent. In another experiment 7^{th} day after withdrawal of treatment, activity of all the enzymes come back to nearly normal. The above study concluded that the Cyperstar is highly toxic to the fish in comparison to Apigenin and Rutin but all are metabolized in body of fish within seven days of withdrawal of treatment.

Key words: Apigenin; *Codiaeum variegatum*; Cyperstar; *Jatropha gossypifolia*; Rutin.

INTRODUCTION

A number of predatory fishes like *Channa* species, *Clarius* species and *wallago attu* are commonly found in Indian aquaculture. Almost 42 fish species of genus *Channa* are available in all over the world. Out of 40, almost four species (*Channa punctatus*, *Channa marulius*, *Channa striatus* and *Channa gachua*) are common in north India [1]. These fishes are predatory in nature and feed upon the fry of cultured carps [2]. Due to faster growth rate, these fishes share and better utilized the cultured carp habitat as well as food also [3], so these fishes adversely affect the aquaculture production and ultimately affect the economy of fish former. Most of the predatory fishes breed in ponds, a little earlier than the spawning time of cultured carps and their fry feed vigorously on available food in the pond, when the spawn of cultured carps released, the young ones of predatory fishes are grown enough to feed upon fingerlings of cultured carps [4].

The weed fishes are uneconomic, small size fish that naturally occur accidently introduced in pond along with carp spawn. The majority of common weed fishes are active competitors with the major carps for the food available in the pond. The presence of predatory and weed fishes in

culture pond is a serious problem for fisherman in Uttar Pradesh, so removal of unwanted fish population from the culture carp ponds in necessary before the seed of cultured carps is added.

Eradication of unwanted fishes were made by poisoning in early 1900, but the use of chemicals as a management of tools being about 1930 and has become well accepted practice in small as well as large scale fish industries due to its convenience of use, broad range of effectiveness and cheapness [5, 6]. Indiscriminate use of these synthetic pesticides, they persist in the water and ultimately affect the non-target organisms especially major carp [7]. Persistent chemical molecules with long half-life periods in chemical pose a threat to fish also to human population consuming the affected fish [8].

The *Cirrhinus mrigala* carp, also known as Indian carp, is a species of ray-finned fish in the carp family. Native to streams and Rivers in India, the only surviving wild population is in the Cauvery River, leading to its IUCN rating as vulnerable. It is widely aqua formed and introduced populations exist outside its native range [9]. Mrigala is fast growing fish and therefore preferred for pisciculture, when cultured artificially can grow to a great size. Rich in protein content as food. Young fingerlings are usually available as fish seed. These can be purchased and then be cultivated as adult stage. This is a more commercial method of fish culture. When culture on commercial scale, it can yield good margin as profit. The cultivation also generates employment in rural areas. It reaches a maximum length of one meter [10].

It is an important aqua cultured freshwater species in south Asia [11]. In the year 2001 alone, 340,741 tones were harvested through aqua forming with India and Bangladesh being the largest producer.

The aim of this study was to measure the comparative toxic effect of synthetic as well as plant origin piscicide, which is use for the removal of unwanted fishes from fish culture pond on non-target Indian major carp *Cirrhinus mrigala*.

2. MATERIALS AND METHODS

Collection of plant materials

The leaves of both the plant *Jatropha gossypifolia*, *Codiaeum variegatum* were collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur and plant was identified by Prof. S. K. Singh, Taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U.P.) India, where the voucher specimen is deposited. The leaves were washed properly by tap water and cut the leaf by scissors and then dried in shady place and finally dried in an incubator at about 35°C temperature, the dried leaves were powdered by electric Grinder.

Collection and maintenance of test animals

Freshwater major carp *Cirrhinus mrigala* (70 ± 2 g body weight; 21 ± 1cm in total length) were collected from Ramgarh Lake of Gorakhpur (U.P) India, the collected fish were maintain in plastic tank containing 100 L of de-chlorinated tap water for acclimatization to laboratory condition for seven days. Water of aquarium aerated continuously by electric aerator and the dead fishes removed from the aquarium to avoid any type of contamination.

Physico-chemical properties of water

The physico-chemical properties of water measured in the beginning of experiment. Temperature 26-28°C, pH 7.2-7.4, dissolved oxygen 6.5 ml/L, carbon dioxide 2-8 mg/L, alkalinity 9.6 ppm hardness of water 140 ppm. All the above parameters are determined by methods of APHA-AWWA-WPCF [12].

Extraction of compounds

The Apigenin and Rutin were isolated from the leaves of *Jatropha gossypifolia* and *Codium variegatum* respectively by the method of Subramanian et al. [13]. The leaves of these plants were washing properly in tap water and cut the leaves by scissors then dried in shady place and finally dried in an incubator at about 35°C temperature; dried leaves were powder by electric Grinder. About 50 g powder of leaves was subjected in Soxhlet extraction unit with about 250-300 ml ethyl alcohol for about 72h at 30-40°C,

For compound Apigenin when extraction was completed then filtered and a little amount of crude yellow powder obtained. After addition of NaOH and HCl, Apigenin was obtained which was crystallized by methanol, Apigenin extracted from

Jatropha gossypifolia leaves were confirmed by UV spectra data of Dordevice et al. [14].

In case of compound Rutin after extraction, the aqueous layer was collected and left to stand in a cold place for 72 hours; a yellow precipitate separated out from the solution. The precipitate was filter and washed with a mixture of chloroform: ethylacetate: ethanol (2:1:1). The un-dissolved part of the precipitate was dissolve in hot methanol and filtered, the filtrate was evaporating to dryness to give 280 mg yellow powder (Rutin), and its melting point was measured as 194-196°C [15].

Treatment condition for toxicity testing

Toxicity experiment was performing according to Singh and Agarwal [16]. Ten experimental major carp *Cirrhinus mrigala* kept in a glass aquarium containing 12L de-chlorinated tap water each, the physicochemical characteristics of experimental water was temperature 28-30°C, pH 7.1-7.3 dissolved oxygen 6.8-7.0 mg/L, carbon dioxide 3.8-6.0 mg/L, alkalinity 105-109 ppm. Aquarium water changed every 24 hour. Fishes were exposing for 24h, 48h, 72h and 96h at four different concentrations (w/v) of Apigenin, Rutin and Cyperstar (synthetic pyrethroid pesticide). Six aquaria were set up for each dose, control animals were kept in similar condition without any treatment. Mortality was recorded after every 24h up to 96h exposure periods. Dead animals were removed to prevent any type of contamination in experimental aquaria. Toxicity data obtained from this study, computed through POLO computer program of Robertson et al. [17].

Estimation of acetylcholinesterase activity

Acetyl cholinesterase (AChE) activity was measure by the method of Ellman et al. [18] as modified by Singh and Agarwal [19]. Tissue (50 mg/ml, w/v) was homogenized in 0.1ml phosphate buffer (pH 8.0) for 5 minutes in an ice bath and centrifuged at 1000 g for 30 minutes at -4°C and supernatant was used for enzyme assay. Taking 0.1 ml homogenate then mixed 2.9 ml of phosphate buffer (pH8.0), 0.1ml 5,5-dithio-bis-nitrobenzoate (DTNB) and 20µL substrate. Mixed cuvette contents by inverting it and absorbance read at 412 nm at every 30 sec time interval. After 3-5 minutes, the reaction rate becomes linear. Enzyme activity has been expressed as µM SH hydrolyzed/min/mg of wet tissue.

Estimation of phosphatase activities

Activities of phosphatase (acid and alkaline phosphatase) were measured according to the method of Anderch and Szcypinski [20] modified by Bergmeyer [21]. Homogenates (50 mg/ml, w/v) were prepared in ice-cold 0.9% saline and centrifuged 5000 g at 4°C for 15 minutes and supernatant was used enzyme source. Supernatant (0.1ml) was incubated with1.0 ml of alkaline buffer substrate solution incubation was carried for 30 minutes at 37°C in case of both the enzymes. The reaction was stop by adding excess of alkali (10.0 ml of 0.02 N NaOH in case of alkaline phosphatase and 4.0 ml of 0.1N NaOH in case of acid phosphatase). The p-nitrophenyl phosphate, gave yellow colour with NaOH. Optical density was measured at 420 nm against blank prepared simultaneously. In the blank tube, enzyme was inactivating by addition of NaOH before mixing with the incubation mixture. Optical density was compare with standards consisting of different concentrations of p-nitrophenol solution. Protein was estimated in1.0 ml of supernatant for each replicate. The enzyme activity was express as amount of p-nitrophenol formed/30 min/mg protein in supernatant.

Statistical analysis

Each experiment was replicate at least six times and data has expressed as mean ±SE. Student's t-test was applied for locating significant differences [22].

Withdrawal experiment

In order to see the effect of withdrawal of treatment of Apigenin, Rutin and Cyperstar (synthetic pyrethrois pesticide) to freshwater major carp *Cirrhinus mrigala* exposed for 24h, 96h of 40% and 80% of LC_{50}. One-half of the animals were scarified for the measurement of the activity of the enzymes acetylcholinesterase (AChE), acid and alkaline phosphatase. The other half animals transferred to Apigenin, Rutin and Cyperstar free water, the water was regularly, changed every 24h for the next 7 days, and then measured the activity of enzyme acetylcholinesterase (AChE), acid and alkaline phosphatase in nerve tissues of the fish.

3. RESULTS

Effects on behavior of fish and poisoning symptoms

In case of herbal compound behaviorally, fishes start scratching their nostril at the bottom of aquarium and firstly came at the water surface for engulfing air. Within 20-30 minutes, fishes try to escape from test aquaria. Within 35 min., their movement was slow down, but they continue to swim water air interface. Thereafter, fishes show irregular, irritating and sometime jerky movement that was increases as exposure period increased. At higher doses after 10-20 hours, loss of body equilibrium, hemorrhage, and salivation, which manifested itself as reddish color on snout, opercular region and finally fishes were died. After exposure of Cyperstar (synthetic Pyrethroid pesticide) also showed behavioral changes, fishes aggregated at one corner of aquarium, and loss of equilibrium, slowly became lethargic, hyperactive excited, restless and secreted excess mucus all over their bodies. The fish exhibited peculiar behavior of trying to leap out from the treated medium, which can be viewed as an escaping phenomenon. Fishes often spiral rolled at intervals, finally sank to bottom with their least operculum movement, and died with their mouth opened.

Dose-mortality response

The LC_{50} values of the aqueous extracts of Apigenin, Rutin of *Jatropha gossypifolia*, *Codiaeum variegatum* respectively and Cyperstar (synthetic Pyrethroid pesticide) periods ranging from 24h or 96h of fish *Cirrhinus mrigala* are shown in Table 1. The toxicity was time as well as dose dependent, as there was a significant negative correlation between LC_{50} values and exposure periods. Thus, the LC_{50} of Apigenin, Rutin and Cyperstar decreased from 83.26 to 44.92 mg/L, 58.43 to 43.62 mg/L 0.009 to 0.005 mg/L, after 24h or 96h exposure respectively to major carp *Cirrhinus mrigala* (Table 1).

The slope values were steep and heterogeneity factor was less than 1.0 indicates that the result found to be within the 95% confidence limits to LC_{50} values. The regression test (t ratio) was greater than 1.96 and the potency estimation test (g value) was less than 0.5 at all probability levels (Table 1).

Acetylcholinesterase activity

Table 2 clearly shows that sub-lethal exposure of Apigenin at 40% and 80% of LC_{50} the AChE activity inhibited 76%, 56% at 24h with respect to control but at 96h exposure the AChE activity also inhibited as 44%, 23% at 40% and 80% of LC_{50} respectively with respect to control. In case of Rutin both 24h and 96h exposures of 40%, 80% of LC_{50} AChE activity also inhibited with respect to control. In case of Cyperstar 24h exposure of 40%, 80% LC_{50} AChE activity inhibited as 63%, 58% respectively with respect to control, same trained was found at 96h exposure of 40% and 80% LC_{50} AChE activity inhibited as 46% and 23% with respect to control.

Phosphatase activity

Table 3 indicates that sub-lethal treatment of Apigenin of 40% and 80% of LC_{50} (24h), alkaline phosphatase activity inhibited by 80%, 60% respectively with respect to control. At long duration (96h) exposure, 40% and 80% of LC_{50} of Apigenin also inhibited the activity of enzyme alkaline phosphatase by 46%, 35% respectively with respect to control, and same trained was found at 96h exposure of LC_{50} of cyperstar, activity of enzyme inhibited as 58% and 55%. In the case of enzyme acid phosphatase, exposure of 40%, 80% LC_{50} of Apigenin, Rutin and Cyperstar also significantly inhibited the enzyme activity at 24h or 96h with respect to control (Table 3).

After seven days in a clean enclosure following exposure to 80%, 96 hour of LC_{50} of Apigenin, Rutin and Cyperstar (Tables 2 and 3) there was significant ($P < 0.05$) recovery observed in the AChE, acid and alkaline phosphatase enzyme in nerve tissue of major carp *Cirrhinus mrigala.*

4. DISCUSSION

It is clear from present study, synthetic pesticide Cyperstar contamination is dangerous to aquatic ecosystems, and this fact should take into consideration when this pesticide is use in agriculture for the control of pest populations. It is also conclude that although plant based piscicide are consider as less toxic and environment friendly, but precautions must be taken when it is used in fish inhabiting areas since the excess application can

affect aquatic life (fish). The stressful breathing behavior exhibited by the fish reflected by increased opercula ventilation with increased concentration of extract may be as result of respiratory impairment due to effect of Apigenin and Rutin on the gills [23, 24].

Table 1. Comparative table of LC values of extracted compound Apigenin, Rutin and synthetic pyrethroid pesticide (Cyperstar), used against major carp *Cirrhinus mrigala* (Nain) at different time interval.

Compounds	Exposure period (in hours)	LC values (mg/L)	Limits		Slope value	Heterogeneity
			LCL	UCL		
Apigenin	**24**	LC_{10}=73.71 LC_{50}=83.26 LC_{90}=99.31	65.44 68.32 88.24	73.15 87.85 102.16	2.28±7.31	0.02
	48	LC_{10}=65.51 LC_{50}=68.56 LC_{90}=89.34	46.77 55.72 65.67	55.63 67.17 82.15	3.95±6.96	0.04
	72	LC_{10}=61.54 LC_{50}=62.11 LC_{90}=81.15	48.75 65.21 74.04	59.34 68.19 84.59	7.32±6.95	0.03
	96	LC_{10}=38.16 LC_{50}=44.92 LC_{90}=62.63	36.86 47.92 59.21	41.33 58.45 67.15	5.47±7.72	0.02
Rutin	**24**	LC_{10}=55.62 LC_{50}=58.43 LC_{90}=73.52	44.54 63.32 64.04	51.82 68.05 75.21	73.34±41.85	0.53
	48	LC_{10}=44.41 LC_{50}=52.32 LC_{10}=61.71	42.91 66.21 71.31	49.65 68.16 80.20	46.15±31.44	0.03
	72	LC_{10}=38.75 LC_{50}=44.40 LC_{90}=52.07	27.73 35.95 46.44	34.07 41.81 52.82	48.91±23.94	0.04
	96	LC_{10}=40.12 LC_{50}=43.62 LC_{90}=51.53	36.45 41.51 48.49	41.21 46.82 53.31	110.13±51.54	0.19
Cyperstar (Pyrethroid pesticide)	**24**	LC_{10}=0.005 LC_{50}=0.009 LC_{90}=0.013	0.001 0.008 0.010	0.006 0.013 0.023	0.56±0.75	0.49
	48	LC_{10}=0.006 LC_{50}=0.007 LC_{90}=0.013	0.0007 0.008 0.019	0.006 0.014 0.028	0.49±0.66	0.76
	72	LC_{10}=0.002 LC_{50} =0.006 LC_{90}=0.0105	0.004 0.003 0.009	0.005 0.008 0.017	0.27±0.40	0.061
	96	LC_{10}=0.0008 LC_{50}=0.005 LC_{90}=0.009	0.0101 0.003 0.007	0.004 0.006 0.002	0.27±0.043	0.273

- Batches of 10 fishes were exposed to four different concentration of extracted compound Apigenin, Rutin and synthetic pyrethroid pesticide (Cyperstar).
- Concentrations given are the final concentrations (w/v) in laboratory conditions.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL = lower confidence limit; UCL = Upper confidence limit.

Table 2. Inhibition of Acetyl cholinesterase (AChE) in the nervous tissue of major carp *Cirrhinus mrigala* (Nain) exposed to sub-lethal doses (40% and 80% LC_{50}) of Apigenin, Rutin and synthetic pyrethroid pesticide (Cyperstar) at 24hrs or 96hrs exposure periods

Name of compounds	Exposure periods (in hours)	AChE activity (µm SH hydrolyzed/min/mg protein)			Recovery after 7^{th} days of withdrawal
		Control	40% of LC_{50}	80 % of LC_{50}	
Apigenin	**24**	0.0072±0.023 (100)	0.055±0.0065* (76)	0.040±0.0005* (56)	0.0064±0.043 (89)
	96	0.0069±0.025 (100)	0.044±0.0012* (45)	0.016±0.0004* (23)	0.0064±0.013 (93)
Rutin	**24**	8.71±0.231 (100)	63.32±3.15* (72)	56.54±0.22* (65)	8.69±0.330 (99)
	96	8.95±1.43 (100)	58.03±0.54* (64)	43.87±0.38* (49)	8.87±1.33 (98)
Cyperstar (Pyrethroid pesticide)	**24**	0.0062±0.015 (100)	0.037±0.054* (63)	0.033±0.012* (58)	0.0056±0.005 (90)
	96	0.0052±0.045 (100)	0.031±0.043* (58)	0.027±0.007* (55)	0.0046±0.002 (88)

- Values are mean ± SE of six replicates.
- Values in parentheses indicate percent enzyme level with controls taken as 100%.
- * Significant ($p<0.05$) when student's't' test was applied between control and treated groups.

In addition, the inability of the gills surface to actively carry out gaseous exchange might be responsible for the recorded mortalities which was shown to be significantly different ($P<0.05$) and directly proportional to the extract concentration and exposure period. The darkened patches observed on the skin could be because of the dispersion response of the melanin pigments in the chromatophores, which move towards the periphery [25].

Plant origin piscicide

Extracted compound Apigenin and Rutin significantly inhibit the activity of enzyme acetylcholinesterase, acid and alkaline phosphatase in brain tissues of major carp *Cirrhinus mrigala* in time as well as dose dependent manner. Effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which affect physiology of body.

Various forms of abnormal behavior were observed in *Heteropneustes foossilis* when exposed to different concentrations of Rutin of *Codiaeum variegatum* [26]. By changing a large number of behavioral responses, fishes try to resist the change in the aquatic environment and minimize the harmful effect of Rutin, these include the change in skin color, start scratching their nostril at the bottom and side of the aquarium and freely came at the water surface for engulfing air [27].

During neurotransmission, ACh is releases from the nerve into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from nerve. AChE, also located on the post-synaptic membrane, terminate the signal transmission by hydrolyzing ACh.

The liberated choline is taken up again and ACh is synthesized by combining with acetyl-CoA through the action of choline acetyltransferase [28]. To receive another impulse, ACh must be released from the ACh receptor. This occurs when the concentration of ACh in the synaptic cleft is very low. Inhibition of AChE leads to accumulation of ACh in the synaptic cleft and results in impeded neurotransmission. AChE-inhibitors block the normal breakdown of the neurotransmitter, acetylcholine into acetic acid and choline. They do it by blocking the site where acetylcholine attaches the enzymes. In normal condition, ACh attaches to the serine hydroxyl group on AChE and Cyperstar may have high affinity to conjugate with serine and form enzyme-inhibitor complex.

This prevents acetylcholine from interacting with cholinesterases enzyme and being break down. This leads to the buildup of excessive levels of neurotransmitter ACh at the neuromuscular junctions. Due to this nerves collapse in brain of

fish that leads behavioral changes [29].

Synthetic piscicides

In the present study, Cyperstar (synthetic Pyrethroid pesticide) shows significant behavioral changes in fish (hyperactive movement, hypomovement, vertical position and loss of equilibrium). Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment. Behavioral action is a sequence of quantifiable actions, which operated through the central and peripheral nervous systems and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life such as feeding, reproduction and predator avoidance [27, 29].

Enzyme alkaline phosphatase plays an important role in animal metabolism. Vorbrodt [30] has reported that the role of this enzyme is in the transport of metabolites across the membrane. The enzyme has been shown to be intimately associated with protein synthesis [31] and is thus involved in the synthesis of certain enzymes [32].

Acid phosphatase is the lysosomal enzyme and plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis [33, 34].

Table 3. Changes in acid and alkaline phosphatase activity in brain tissues of fish *Cirrhinus mrigala* (Nain) exposed to sub-lethal doses (40% and 80% of LC_{50}) of Apigenin, Rutin and synthetic pyrethroid pesticide (Cyperstar) at 24hrs and 96hrs exposure periods.

Name of compounds	Exposure periods (in hours)	Alkaline phosphatase activity as (µm *p*-nitrophenol formed/30 min/mg protein)			Recovery after 7th days of withdrawal
		Control	**40% of LC_{50}**	**80 % of LC_{50}**	
Apigenin	**24**	0.0036±0.014 (100)	0.028±0.0002* (80)	0.021±0.0003* (60)	0.0033±0.011 (92)
	96	0.0038±0.011 (100)	0.017±0.0005* (46)	0.013±0.0002* (35)	0.0034±0.016 (89)
Rutin	**24**	0.0035±0.0012 (100)	0.022±0.0003* (64)	0.022±0.0011* (63)	0.0030±0.0112 (86)
	96	0.0039±0.0002 (100)	0.014±0.0006* (37)	0.015±0.0005* (43)	0.0035±0.024 (90)
Cyperstar (Pyrethroid pesticide)	**24**	0.0027±0.0005 (100)	0.018±0.0004* (65)	0.0176±0.0007* (63)	0.0023±0.0255 (85)
	96	0.0034±0.0009 (100)	0.022± 0.0003* (58)	0.016± 0.0004* (48)	0.0030± 0.0149 (88)
		Acid phosphatase activity as (µm *p*-nitrophenol formed/30 min/mg protein)			
		Control	**40%of LC_{50}**	**80 % of LC_{50}**	
Apigenin	**24**	0.0025±0.0005 (100)	0.019±0.0002* (76)	0.018±0.0003* (72)	0.0022±0.008 (88)
	96	0.0028±0.006 (100)	0.012±0.0003* (46)	0.010±0.0005* (38)	0.0025±0.003 (99)
Rutin	**24**	0.0029±0.011 (100)	0.018±0.0012* (65)	0.016±0.0023* (58)	0.0026±0.013 (90)
	96	0.0023±0.008 (100)	0.008±0.0005* (38)	0.008±0.0006* (35)	0.0020±0.0015 (87)
Cyperstar (Pyrethroid pesticide)	**24**	0.0032±0.002 (100)	0.018±0.0014* (63)	0.016±0.0013* (58)	0.0028±0.006 (88)
	96	0.0035±0.004 (100)	0.016±0.0005* (44)	0.014±0.0005* (43)	0.0030±0.013 (86)

- Values are mean ± SE of six replicates.
- Values in parentheses indicate percent enzyme level with controls taken as 100%.
- * Significant ($p<0.05$) when student's 't' test was applied between control and treated groups.

Any change on acid and alkaline phosphatase activity can feel the metabolism of the fish.

Comparison of the LC_{50} values clearly indicates that the plant-based piscicide is less toxic compared to the synthetic one. To reduce the chemical load on the environment, it is suggested that use of plant-based pesticides should be encouraged. However, care should be taken to use even the plant-based piscicide at moderate levels. It is advocated that more and more plant products should be developed with proper and targeted action and this eventually helps in keeping the environment free from hazardous chemicals.

AUTHORS' CONTRIBUTION

BP: conception and design; AS: development of methodology; BP: Acquisition of data; BP and AS: Writing, revised and revision of the manuscript; Administrative, technical or material support; AS: study supervision. Authors are involved in drafting the manuscript, read and approved the final manuscript.

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TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Preliminary survey of avifaunal diversity around Shetrunji River, Dhari, India

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ABSTRACT

The present study was carried out to document of the aquatic avifaunal diversity of Shetrunji River, Dhari located in the Amreli District of Gujarat State. A total of 18 species of birds belonging to 8 families viz. Ciconiidae, Phoenicopteridae, Phalacrocoracidae, Podicipedidae, Anatidae, Ardeidae, Threskiornithidae and Alcedinidae were recorded in the study area from October 2013 to December 2013. 14 species of these were residents and 2 were residents with local migrants. Ardeidae was highest in dominance family followed by Anatidae, Ciconiidae and Threskiornithidae, where as in concern of the order Ciconiiformes was highest in dominance of species followed by Anseriformes. During winter the maximum birds were observed from recorded species due to easy accessibility of food. Among all the bird species recorded in this area, 16 were least concern and 2 species of birds such as Black Headed Ibis (*Threskiornis melanocephalus*) and Painted Stork (*Mycteria leucocephala*) are Near Threatened under Red List category of IUCN 2013.

Key words: Aquatic; Avifauna; Diversity; Status; River.

INTRODUCTION

The present study is carried out to identify the status of avifauna at Shetrunji River, Dhari located in the Amreli District of Gujarat state. Diversity of avifauna is one of the most important ecological indicators to evaluate the quality of habitats. Nowadays, avifaunal diversity has been decreasing due to the destruction of natural habitats and human disturbances [1]. About 9,000 living species of birds in the world and more than 1250 species in India are known at present. 25 to 30 avian orders are recognized depending on the taxonomists. According to [2], there are 34 orders out of them 27 orders of living birds of which two recently became extinct, and 7 orders belonging to fossil birds. Birds are essential animal group of an ecosystem and maintain a trophic level. Therefore, detail study on avifauna and their ecology is important to protect them. Birds play prominent and diverse role in religion, and popular culture. They have their functional role in the ecosystem as potential pollinators and scavengers and are rightly called as bio-indicators [1].

Birds are important group of aquatic food chain. They feed on vegetation, fishes and other animals of the reservoir, the birds like Kingfisher, Stork, Cormorants and Herons feed on fishes, frogs, tadpoles and terns feed in deeper waters [3]. Birds occupy an important position in the animal kingdom, especially in relation to man. Today due to industrialization and urbanization most of the water bodies in India are severely polluted, which gives stress on the physical, chemical and biological

characteristics of the water. The life of aquatic birds depends directly on physical and chemical properties of aquatic environment. It is our solemn duty to protect and nurture this precious gift of nature [3]. The avian species richness in any area is largely due to presence of water bodies [4] and is essential on building sound conservation programs for water bird assemblages [5]. During the last few decades considerable studies on avifauna diversity from different freshwater bodies of India have been carried out by researchers like, Bhadja and Vaghela [1], Donar et al. [3], Osmatston [6], Ali [7], Kannon [8], Davidar [9], Jhingram [10], Ghazi [11], Mujumdar [12], Newton et al. [13], Ghosal [14], Rathore and Sharma [15], Yardi et al. [16], Kulkarni et al. [17], and Kumar [18]. However very little information is available about status of aquatic avifaunal status of Saurashtra, Gujarat. Therefore, the present study provides a comprehensive check-list of birds to identify the status of aquatic avi-faunal diversity of Shetrunji River, Dhari located in the Amreli District of Gujarat State.

2. MATERIALS AND METHODS

Study Location

The present study of aquatic avifauna was studied from October 2013 to December 2013 at Shetrunji River, Dhari located in the Amreli District of Gujarat state (Figure 1). Dhari is a town in Amreli district, Saurashtra. Dhari is located on the banks of river Shetrunji on which the famous Khodiyar Dam is located. Shetrunji River is a north-flowing river in western India in Gujarat.

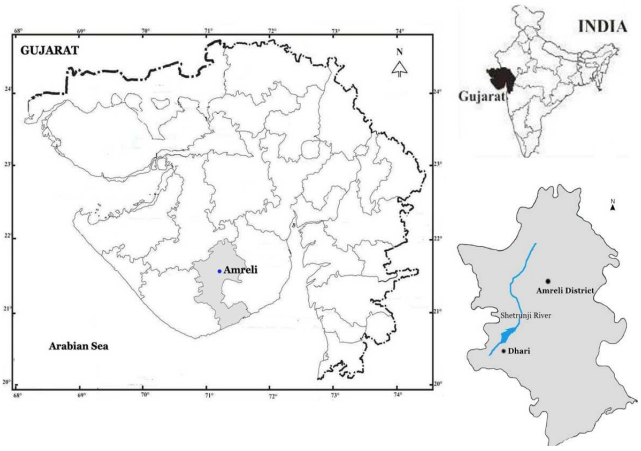

Fig. 1. Map showing the study area, Shetrunji River, Dhari, Amreli district, Saurashtra.

It rises northeast of the Gir Hills, near Dhari in Amreli district [19]. The Amreli district experiences a semi-arid climate, summers are very hot and dry and the monsoon is accompanied by moderate rainfall.

Methodology

The identification and classification of bird was based on the standard data as prescribed by [20-22]. Birds sighted during the study period were categorized according to their status as residents (R), some birds sighted occasionally during specific season of study period, which are not resident of study area, are included as migrant birds (M). Systematic list and status of the birds was constructed on the basis of each taxon according to Sarker and Sarker [23] and Hossain et al. [24]. All observations were made using binocular (10 X 50) magnification while walking on the boundary of the selected sampling sites. Photographic documentation was done with Fujifilm FinePix Semi-SLR Cameras.

3. RESULTS AND DISCUSSION

A total of 18 species were recorded belonging to 8 families during the study Period (Table 1). The families with number of birds species observed are Ardeidae 6, Anatidae 4, Threskiornithidae 2, Ciconiidae 2, Alcedinidae 1, Phalacrocoracidae 1, Phoenicopteridae 1 and Podicipedidae 1 (Table 1; Figure 2).

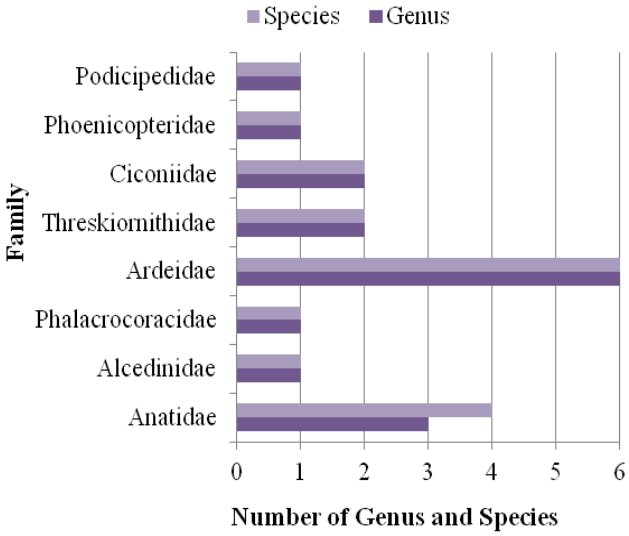

Fig. 2. Family wise number of genus and species recorded from the study area.

Table 1. Avifaunal species recorded at study sites near Shetrunji River, Dhari, Amreli District during October 2013 to December 2013 (M - Migratory; R - Resident; RM - Residential Migratory).

No.	Common Name	Scientific Name	Status
	Family: Anatidae		
1	Spoted Bill Duck	*Anas poecilorhyncha* Forster, 1781	RM
2	Northern Shoveler	*Anas clypeata* Linnaeus, 1758	M
3	Whistling duck	*Dendrocygna arcuata* Horsfield, 1824	R
4	Knob-billed Duck	*Sarkidiornis melanotos* Pennant, 1769	
	Family: Alcedinidae		
5	Lesser Pied Kingfisher	*Ceryle rudis* Linnaeus, 1758	R
	Family: Phalacrocoracidae		
6	Little Cormorant	*Phalacrocorax niger* Vieillot, 1817	R
	Family: Ardeidae		
7	Little Egret	*Egretta garzetta* Linnaeus, 1766	R
8	Intermediate Egret	*Mesophoyx intermedia* Wagler, 1827	R
9	Grey Heron	*Ardea cinerea* Linnaeus, 1758	R
10	Striated Heron	*Butorides striata* Linnaeus, 1758	R
11	Cattle Egret	*Bubulcus ibis* Linnaeus, 1758	R
12	Indian Pond Heron	*Ardeola grayii* Sykes, 1832	R
	Family: Threskiornithidae		
13	Black Headed Ibis	*Threskiornis melanocephalus* Latham, 1790	R
14	Eurasian Spoonbill	*Plataiea leucorodia* Linnaeus, 1758	R
	Family: Phoenicopteridae		
15	Greater Flamingo	*Phoenicopterus ruber* Linnaeus, 1758	RM
	Family: Podicipedidae		
16	Little Grebe	*Tachybaptus ruficollis* Pallas, 1764	R
	Family: Ciconiidae		
17	Asian Openbill Stork	*Anastomus oscitans* Boddaert, 1783	R
18	Painted Stork	*Mycteria leucocephala* Pennant, 1769	R

Among all the species recorded 14 species were residential, 2 species were residential migratory and 1 species belonging to family Anatidae was migratory. However most of the species were local movement for their food resources. Some of the aquatic birds observed in the present study were grouped into egret, cormorants, herons, ducks and king fisher as shown in the figures (Figure 2). The species Grey Heron, Little Egret, Open Bill Stork were carnivorous; they were found feeding upon aquatic insects and their larvae, molluscs, crustaceans, fishes, tadpoles, frog etc [3]. The species such as Little Cormorant, Pied King Fisher were Piscivorus; their food consisted of fish species [1, 3, 25-27]. The occurrence of these birds in the area suggests that the area provides a favorable condition for the bird's breeding, feeding and nesting. Studies have shown that birds migrate to different areas because of seasonal changes [28], availability of food [29] and threat of predation [30]. The factors responsible for the decline in population of aquatic birds are due to extensive utilization of water for domestic purposes, unlimited fishing, utilization of its marshy vegetation for grazing of livestock and decrease in rainfall [29-31]. Water pollution due to agriculture run off,

influx of sewage, industrial waste is additional threats to this reservoir [1, 3]. Among all the bird species recorded in this area, 16 were least concern (LC) and 2 species of birds such as Black Headed Ibis (*Threskiornis melanocephalus*) and Painted Stork (*Mycteria leucocephala*) are Near Threatened (NT) under Red List category of IUCN 2013 [32] (Table 2).

Table 2. Check list of aquatic bird species with their threatened status according to IUCN Red List of Threatened Species (TS = Threatened Status: NT = Near Threatened and LC = List Consent).

Order	Family	Common Name	Scientific Name	TS
Anseriformes	Anatidae	Spoted Bill Duck	*Anas poecilorhyncha*	LC
		Northern Shoveler	*Anas clypeata*	LC
		Whistling duck	*Dendrocygna arcuata*	LC
		Knob-billed Duck	*Sarkidiornis melanotos*	LC
Coraciiformes	Alcedinidae	Lesser Pied Kingfisher	*Ceryle rudis*	LC
	Phalacrocoracidae	Little Cormorant	*Phalacrocorax niger*	LC
Ciconiiformes	Ardeidae	Little Egret	*Egretta garzetta*	LC
		Intermediate Egret	*Mesophoyx intermedia*	LC
		Grey Heron	*Ardea cinerea*	LC
		Striated Heron	*Butorides striata*	LC
		Cattle Egret	*Bubulcus ibis*	LC
		Indian Pond Heron	*Ardeola grayii*	LC
	Threskiornithidae	Black Headed Ibis	*Threskiornis melanocephalus*	NT
		Eurasian Spoonbill	*Plataiea leucorodia*	LC
	Ciconiidae	Asian Openbill Stork	*Anastomus oscitans*	LC
		Painted Stork	*Mycteria leucocephala*	NT
Phoenicopteriformes	Phoenicopteridae	Greater Flamingo	*Phoenicopterus ruber*	LC
Podicipediformes	Podicipedidae	Little Grebe	*Tachybaptus ruficollis*	LC

Table 3. Order and family-wise number of genus and species of avifauna recorded from the sampling site.

Order	Family	Genus	Species
Anseriformes	Anatidae	3	4
Coraciiformes	Alcedinidae	1	1
	Phalacrocoracidae	1	1
Ciconiiformes	Ardeidae	6	6
	Threskiornithidae	2	2
	Ciconiidae	2	2
Phoenicopteriformes	Phoenicopteridae	1	1
Podicipediformes	Podicipedidae	1	1

AUTHORS' CONTRIBUTION

Both authors are involved in drafting the manuscript, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Field evaluation of vermicompost and selective bioinoculants for the improvement of health status of tomato plants

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ABSTRACT

The effect of a combination of known beneficial microorganisms and vermicompost was studied on growth enhancement and yield parameters of two varieties of tomato. Microorganisms used were *Bacillus pumilus*, *Glomus mosseae and Trichoderma harzianum*. Vermicompost was prepared using cow dung and aquatic weeds i.e., *Eichhornia* using earthworms (*Eisenia fetida*). The experimental results showed significant variations in plant growth and yield according to the different treatments. The growth parameters such as plant height, number of fruits were observed at 15 days interval form the date of transplanting seedlings to the field. Maximum enhancement of growth and yield was observed in tomato plants treated with vermicompost alone followed by vermicompost along with all microorganisms and then vermicompost + plant growth promoting bacteria (PGPR). Biochemical components of leaves such as total soluble protein, phenols and chlorophyll as well as antioxidants such as ascorbate and carotenoids were also enhanced to varying degrees by the treatments. Besides, lycopene content of fruits also increased by different treatments. Results clearly indicate that vermicomposting alone or with additional microorganisms not only increase growth and yield but also enhance the quality parameters.

Key words: Vermicompost; Plant growth promoting bacteria (PGPR); Mycorrhizal inoculation (VAM); *Trichoderma*; Tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in the world. Total worldwide production is 152.9 million ton valued at $74.1 billion. In spite of increased production due to the use of hybrid varieties and fertilizer applications, organic vegetables in general, including tomato are much sought after. Using organic compost or vermicompost to enhance production rate of tomato and other vegetable crops is now in general practice. Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. The process is faster than composting; because the material passes through the earthworm gut, a significant but not yet fully understood transformation takes place, whereby the resulting earthworm castings (worm manure) are rich in microbial activity and plant growth regulators, and fortified with pest repellence attributes as well. In short, earthworms, through a type of biological alchemy, are capable

of transforming garbage into 'gold'. Vermicompost contains most nutrients in plant - available forms such as nitrates, phosphates and exchangeable calcium and soluble potassium [1]. There is accumulating scientific evidence that vericompost can influence the growth and productivity of plants significantly [2]. Various greenhouse and field studies have examined the effects of a variety of vermicompost on a wide range of crops including cereals and legumes [3], vegetables [4-7], ornamental and flowering plants [7] and field crops [8]. Annual application of adequate amounts of some organic residues (vermicompost) led to significant increase in soil enzyme activities [9]. Plant growth promoting bacteria (PGPB) directly stimulate growth by nitrogen fixation [10], solubilization of nutrients [11], production of growth hormones, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase [12] and indirectly by antagonizing pathogenic fungi, or by production of siderophores, chitinase, antibiotics, fluorescent pigments and cyanide [10]. The main aim of this research was to determine the effects of vermicompost alone and in combination with PGPR (Plant growth promoting rhizobacteria), AMF (arbuscular mycorrhizal fungi) and BCA (biocontrol agent) on the growth, yield and fruit quality of tomato under field conditions.

2. MATERIALS AND METHODS

2.1. Plant material

Seeds of two varieties of tomato (*Solanum lycopersicum* L.): Romeo and Epoch obtained from local market were selected. These were surface sterilized with 0.1% $HgCl_2$, washed thrice with sterile distilled water and then sown as per experimental design.

2.2. Preparation of vermicompost

For preparation of vermicompost, at first 15-20 cm layer of organic waste material, such as dried aquatic plants was spread on the composting bed then cow dung slurry was sprinkled. *Eisenia fetida* was then added to the vermin bed. The top of the vermin bed was then sealed with cow dung or soil. The vermin bed was stirred often to release organic gas which was produced during the composting process and for proper mixing of the layered materials. The vermicompost was ready in about 40-45 days. The processed vermicompost was black, light in weight and free from bad odor. When the compost was ready, earthworms were separated from it and the manure was dried and sieved for use.

2.3. Bioinoculants, inoculation and growth assessment

The bioresources selected for the present investigations were *Bacillus pumilus* (PGPR), *Glomus mosseae* (AMF) *and Trichoderma harzianum* (BCA). *T. harzianum* (T480) isolated from rhizosphere soil of tea of growing hill region was mass multiplied in wheat bran medium. In case of PGPR isolates, *Bacillus pumilus* (T382) initially isolated from tea rhizosphere was used for seed treatment and inoculation. The bacterial isolates were cultured in Nutrient Broth medium and aqueous suspensions were prepared from cell cultures. Another bioinoculant used was *Glomus mosseae* (AMF); in this case spores of AM fungi were obtained following wet sieving and decanting method [13] from the rhizosphere soil of mature tea plants grown in field conditions.

The field experiment was conducted with complete randomized block design with two varieties of tomato with three replicating rows. Each plot area was 9 ft x 7ft (divided into nine equal rows) and control area was 12ft x 7ft. Surface sterilized tomato seeds were sown in soils treated with BCA (500 g/ row). AMF spore mass was also inoculated in the soil prior to the seed sowing; the same amount of spore was again inoculated to the rhizosphere of 20 d old seedlings four times at an interval of 20 days. In case of PGPR treatment, 1 L of aqueous bacterial suspensions at a concentration of 10^6 cfu/ml was applied as a soil drench to each row containing 20 days old seedlings of tomato four times at an interval of 20 days. All the treatments were carried out singly as well as in combinations, with three replicate rows containing 12 plants per row. Growth assessment was on the basis of parameters including plant height, number of branches, root shoot biomass and yield which were recorded

at definite time intervals.

2.4. Biochemical analyses of leaves

2.4.1. Determination of total soluble protein

Protein was extracted from the tomato leaf using phosphate buffer (pH7.2) and protein content was determined following the method as described by Lowry et al. [14] using BSA as standard.

2.4.2. Extraction and estimation of phenol

One gram of leaf tissue was cut into small pieces and immersed in boiling alcohol (100%) in water bath and heated for 5-10 mins. Tissue was then crushed using 80% alcohol and filtered in Whatman no. 1 filter paper in dark and phenol content was determined following the method as described by Mahadwan and Sridhar [15] using caffaic acid as standard.

2.4.3. Extraction and estimation of chlorophyll

Extraction of chlorophyll was done by homogenizing 0.2 g sample in 80% acetone. The homogenate was filtered through Whatman no. 1 filter paper and the volume was made up to 10 ml. Chlorophyll was quantified from absorbances noted at 645 and 663 nm using Arnon's formula:

Total chlorophyll = $[(20.2\ A_{645}) + (8.02\ A_{663})]$ µg/ml.

2.4.4. Determination of ascorbic acid content

Plant materials were homogenized in cold mortar pestle (using ice) with 10 ml of 6% T.C.A. The filtrate was then extracted (at 0^0 C) 2 ml of 2% DNPH and 1 drop of 10% thiourea then added to 4 ml of extracts. Mixture kept in boiling water bath for 15 min and cooled down by keeping in ice at 0^0 C, 5 ml of 80% H_2SO_4 added to it at 0^0 C. Quantitative estimation of ascorbic acid was carried out following the method as described earlier [16], and using a standard curve of ascorbic acid.

2.4.5. Extraction and estimation of carotenoid

Carotenoid were extracted and estimated according to the method given by Litchtenthaler [17]. Extraction of carotenoids was done by homogenizing 1 g of the sample in methanol. The homogenate was filtered through Whatman no. 1 filter and the volume was made up accordingly. Absorbance of the filtrates was determined at 480 nm, 645 nm, and 663 nm, in UV-VIS spectrophotometer and the carotenoid content was calculated.

2.5. Estimation of lycopene content in tomato fruits

Lycopene was extracted from the tomato using acetone by making it pulp with the help of mortar and pestle and then the 5-10 g of the pulp extracts was transferred to a separating funnel containing about 20 ml of petroleum ether and mixed gently. 20 ml of 5% sodium sulphate solution was added and shaken gently, followed by addition of same volume of petroleum ether. The two phases were separated and the lower aqueous phase was re-extracted until the aqueous phase was colourless. The petroleum ether extracts were pooled out and washed once with a little distilled water. The washed petroleum ether extract containing carotenoid was poured into a brown bottle and kept aside for 30 min. Then the petroleum ether extract was decanted into a 100 ml volumetric flask through a funnel containing cotton wool. The sodium sulphate slurry was washed with petroleum ether until it was colourless and washings were also transferred to the volumetric flask. The volume was made up and the absorbance was measured in a spectrophotometer at 503 nm using petroleum ether as blank. Quantification was done on the basis of this absorbance using the formula:

Absorbance (1 unit) = 3.1206 µg lycopene/ml.

2.6. Observation of root colonization by AMF

Roots were cut into 1 cm small pieces and washed under tap water and boiled in 2% NaOH in hot water bath for 1 h. NaOH was then decanted and washed with tap water for 2-3 times. 1% HCl was added in the sample and kept for 30 mins after which it was decanted and washed thrice with tap water. It was stained with lactophenol-cotton blue (cotton blue: lactic acid: glycerol = 1:1:1) and observed under bright field microscope.

3. RESULTS

3.1. Growth and yield parameters of tomato plant

Plant growth in terms of height and number of fruits per plant was recorded at 15 days interval from the date of transferring seedlings to the experimental plot. Results revealed that growth was affected by the different treatments such as vermicompost, PGPR, BCA, VAM. Maximum growth was observed in plants treated with a combination of all 4 treatments - vermicompost +PGPR+BCA+VAM in variety Romeo and in case of variety Epoch plants treated with vermicompost + PGPR showed maximum growth (Fig. 1).

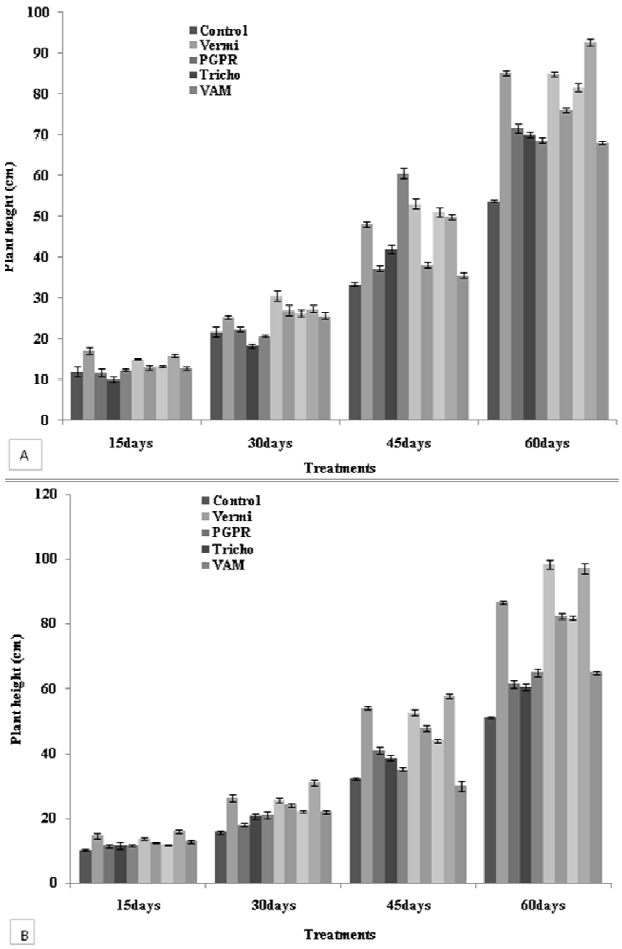

Fig. 1. Effect of different treatments on growth of 2 varieties of tomato. A - Romeo and B - Epoch.

Similarly, wet and dry mass of plants were also enhanced by application of the various treatments (Table 1). In case of yield, recorded after harvesting, vermicomposting along with PGPR or BCA gave highest yield in terms of number of fruits in the two tested varieties. Maximum yield in terms of weight of fruits/plant was observed in the combination of all treatments (Fig. 2).

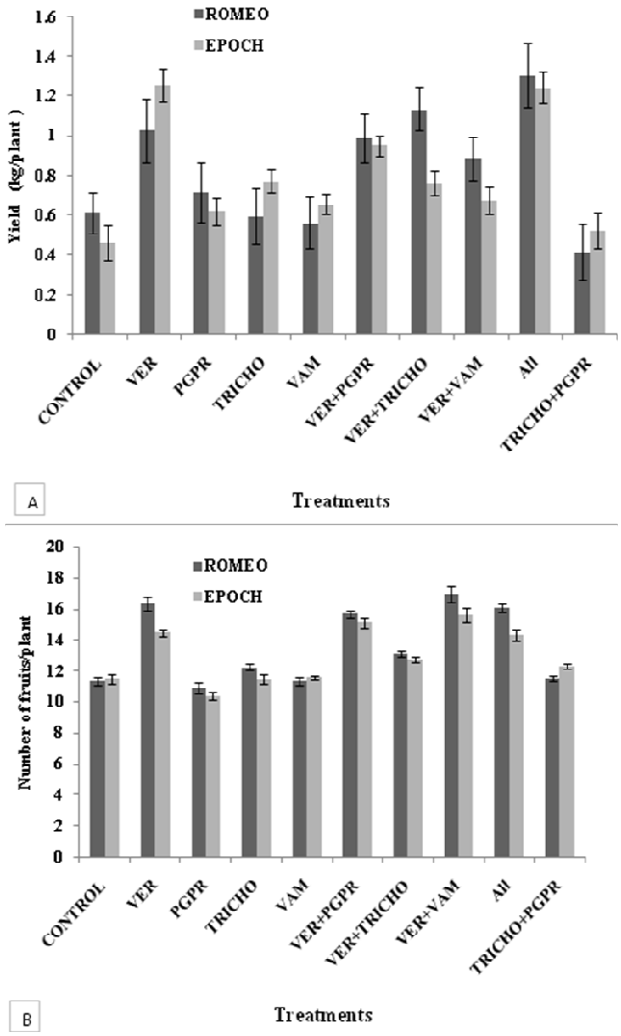

Fig. 2. A - yield of tomato in plants after treatments and B - number of fruits per plant.

3.2. Effect of different treatments on biochemical components of tomato plants

Proteins, phenols and chlorophyll

Estimation of protein contents in both the tomato varieties following various treatments revealed enhanced in protein content following different treatments, of which highest accumulation was obtained in treatments containing all components. Maximum protein content in both

the varieties ranged between 6.5-7.5 mg/g tissue (Table 2).

Quantification of total phenols showed variations not only due to treatments but varietal differences were also observed. Highest amount of total phenol was obtained in plants treated with VAM in Romeo variety and plants treated with vermicompost in Epoch variety (Table 2). In case of chlorophyll content, results revealed that here also maximum accumulation occurred in treatment with vermicompost alone or with addition of VAM (Table 3).

Table 1. Wet and dry biomass of tomato plants per plot.

TREATMENTS	ROMEO		EPOCH	
	Wet Biomass (kg)	**Dry Biomass (kg)**	**Wet Biomass (kg)**	**Dry Biomass (kg)**
CONTROL	4.61±0.73	2.03±0.08	6.03±0.26	2.50±0.02
VERMI	3.30±0.15	1.33±0.08	4.30±0.17	2.40±0.05
PGPR	3.16±0.09	1.53±0.03	2.53±0.08	0.93±0.03
TRICHO	4.26±0.18	1.86±0.06	5.06±0.17	1.03±0.08
VAM	3.46±0.12	1.30±0.05	2.61±0.06	1.27±0.06
VERMI+PGPR	3.76±0.14	1.56±0.12	3.53±0.11	0.96±0.03
VERMI+TRICO	4.83±0.44	1.55±0.65	3.43±0.07	1.34±0.02
VERMI+VAM	4.50±0.26	1.53±0.08	3.69±0.11	1.38±0.06
VERMI+PGPR+ TRICHO+VAM	5.35±0.37	1.66±0.12	4.58±0.06	2.16±0.08
TRICHO+PGPR	2.96±0.03	2.00±0.11	2.03±0.88	0.86±0.03

Vermi = vermicompost; Tricho = *Trichoderma*; VAM = vesicular arbuscular mycorrhizal fungi; PGPR = Plant Growth Promoting Rhizobacteria.

Table 2. Total phenol and total soluble protein content of tomato leaf.

TREATMENTS	ROMEO		EPOCH	
	TOTAL PHENOL (mg/g tissue)	**PROTEIN (mg/g tissue)**	**TOTAL PHENOL (mg/g tissue)**	**PROTEIN (mg/g tissue)**
CONTROL	03.08±0.54	01.41	03.16±0.44	01.72
VERMI	08.50±0.14	03.32	07.91±0.22	04.07
PGPR	03.33±0.22	06.18	05.08±0.22	05.50
TRICHO	01.73±0.27	05.94	02.91±0.30	05.52
VAM	14.25±1.08	04.11	08.23±0.70	03.68
VERMI+PGPR	05.08±0.36	05.09	03.88±0.36	05.21
VERMI+TRICHO	02.15±0.21	03.43	02.83±0.44	03.33
VERMI+VAM	01.33±0.83	06.60	01.91±0.22	06.93
VERMI+PGPR+TRICHO+VAM	11.58±0.68	07.60	07.75±0.38	06.75
TRICHO+PGPR	07.08±0.74	03.40	06.25±0.14	04.70

Vermi = vermicompost; Tricho = *Trichoderma*; VAM = vesicular arbuscular mycorrhizal fungi; PGPR = Plant Growth Promoting Rhizobacteria.

Table 3. Effect of different treatments on chlorophyll contents in tomato leaf.

TREATMENTS	ROMEO			EPOCH		
	Chlorophyll content (mg/g tissue)					
	Chl a	Chl b	Total chl	Chl a	Chl b	Total chl
CONTROL	0.63	0.53	1.16	0.30	0.34	0.64
VERMICOMPOST	1.14	0.48	1.77	0.31	0.43	0.74
PGPR	0.71	0.35	1.06	0.3	0.31	0.61
TRICHODERMA	1.02	0.55	1.57	0.31	0.40	0.71
VAM	1.04	0.55	1.60	0.29	0.27	0.57
VERMI+PGPR	1.11	0.58	1.70	0.30	0.42	0.73
VERMI+TRICHO	0.81	0.39	1.20	0.30	0.38	0.69
VERMI+VAM	0.84	0.39	1.23	0.30	0.45	0.77
VERMI+PGPR+TRICHO+VAM	0.85	0.56	1.41	0.30	0.37	0.69
TRICHO+PGPR	1.05	0.56	1.62	0.30	0.37	0.68

Chl = Chlorophyll; Vermi = vermicompost; Tricho = *Trichoderma*; VAM = vesicular arbuscular mycorrhizal fungi; PGPR = Plant Growth Promoting Rhizobactria.

3.3. Effect of treatments on antioxidant accumulation

3.3.1. In leaves

The major proportion of antioxidant present in tomato is ascorbic acid or "vitamin C". The content of ascorbic acid was estimated at 30 days and 60 days intervals after sowing the two varieties of tomato seedlings in the field. At both periods, results showed highest ascorbic acid content in both the varieties following combination of all four treatments. Quantity of ascorbic acid had almost doubled during 60 days in comparison to 30 days (Fig. 3). Similar results were also obtained in case of carotenoids in Romeo variety but in Epoch highest accumulation was observed PGPR + BCA treatments (Fig. 4).

3.3.2. In fruits

Since tomato fruits are known to accumulate lycopene, this was estimated from the fruits and it was revealed that highest amount of lycopene was present in the fruit of Romeo variety treated with vermicompost and Epoch variety treated with vermicompost and VAM (Fig. 5).

3.4. Root colonization by VAM

The percentage of mycorrhizal colonisation varied significantly under all the treatments compared to the control. The highest root colonization was obtained by mycorrhizal inoculation (VAM), whereas the control treatment (C) gave the lowest colonisation percentage.

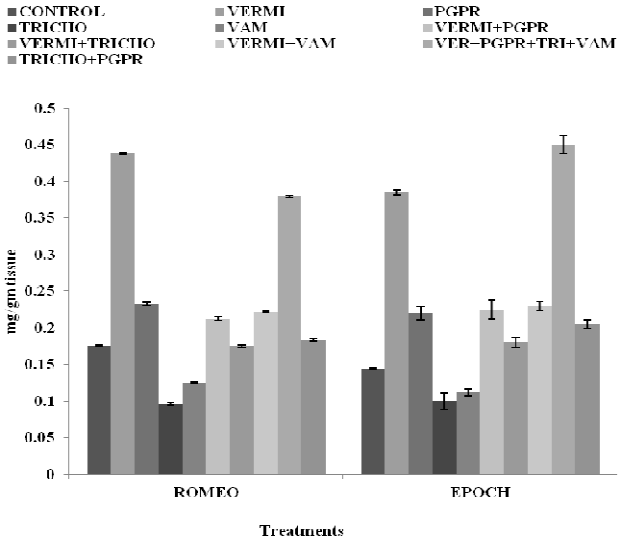

Fig. 3. Ascorbic acid contents of leaves of 2 varieties of tomato following different treatments.

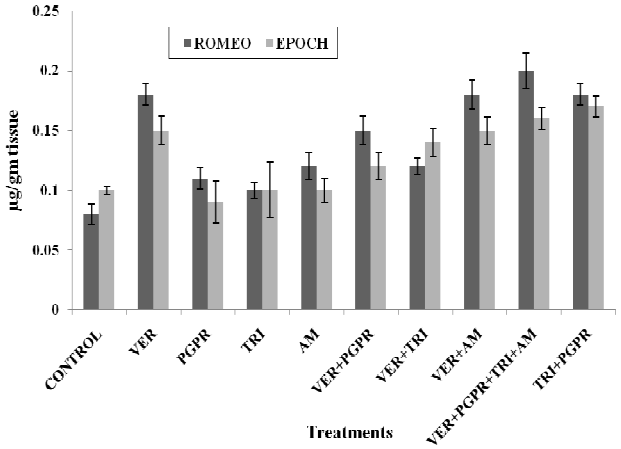

Fig. 4. Effect of different treatments on carotenoid contents in leaves of 2 varieties of tomato.

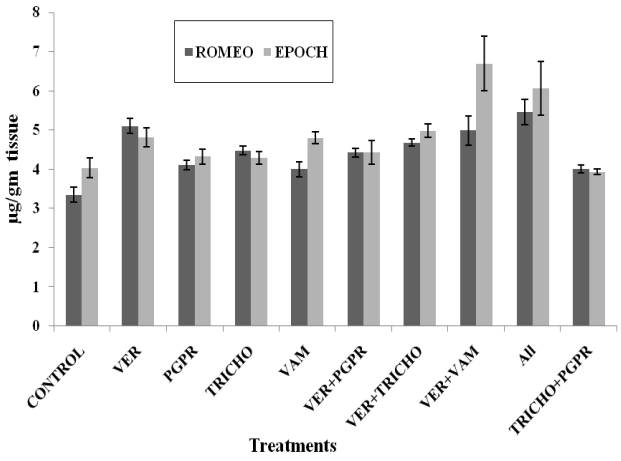

Fig. 5. Lycopene contents of tomato fruits after different soil treatments.

4. DISCUSSION

In the present study it was observed that the application of vermicompost in addition with other bioinoculants in tomato promoted growth and yield of plant. The present report is in agreement with the reports of Gandhi and Sivagama Sundari [18] who found that the availability of macronutrients and micronutrients in different types of vermicompost enhanced plant growth and yield in brinjal (*Solanum melongena* L.). In the present study using different treatments, it was found that highest yield of tomato was obtained in plants where a combination of the different treatments i.e. vermicompost, *Bacillus pumilus*, *Trichoderma* and *Glomus mosseae* were used. Similar results were also previously reported by previous workers [19] in their experiment on productivity enhancement and nematode management through vermicompost and biopesticides in brinjal. Increased leaf area and biomass in various plants following vermicomposting have been reported by some researchers [20, 21] which are in agreement with findings of current study.

Ascorbic acid content, when tested was found in highest amount in plant treated with vermicompost alone and along with other three bioinoculants (PGPR, VAM, *Trichoderma*) in comparison to control set of plant. Same result was found in case of chlorophyll content. It was previously reported that vermicompost may affect different aspects of plant biochemical processes [22, 23]. They significantly promoted contents of vitamin C, phenols and flavonoids in the vermicompost treated plants has been recorded [21].

Another antioxidant compound i.e. lycopene was also found in increased amount in plants treated with vermicompost or in combination with VAM. The results of our study agreed with the previous findings where similar results on the effects of soil amendments on the nutritional quality of okra *(Abelmoschus esculentus* [L.] Moench) were obtained [24].

When root colonization was studied, it was observed that colonization percentage were higher in treated plants rather than control set. Histopathology of the roots also revealed the presence of hyphae, vesicles and arbuscules showing successful colonization of AM fungi within the roots of host plant. Predominant AMF species was studied, where presence of *Glomus* sp. was dominant. The result of this experiment agreed with the previous findings of Osonubi *et al.* [25], who stated that inoculated leguminous woody seedlings had higher mycorrhizal root infections than the non-inoculated seedlings.

Vermicompost is one of the best organic fertilizer which can be used for increasing crop yield without creating any envioronmental hazard. The soil enriched with vermicompost provides additional substances that are not found in chemical fertilizers [8]. However, Ievinsh [26] reported that excessive vermicompost substitutions may adversely influence plant growth, development and yield, especially at germination and seedling stages, and it must be used cautiously for the

agricultural and horticultural activities. It is thus evident from our study that use of vermicompost alone or with other bioinoculants would be eco-friendly and contribute to sustainable agriculture. Besides, farmers would also be benefitted economically. In conclusion it can be said that application of vermicompost in addition with other microorganisms such as *Bacillus pumilus*, *Trichoderma harzianum*, and *Glomus mosseae* increased growth, yield and other quality parameters of tomato plants.

AUTHORS' CONTRIBUTION

BNC and UC are the investigators of the project and the paper was conceptualized by them. PB was the research scholar who carried out the work and wrote the paper. Detailed corrections and paper editing were done by UC and BNC. All authors read and approved the final manuscript.

TRANSPARENCY DECLARATION

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ORIGINAL ARTICLE

Influence of temperature on the fecundity and acetylcholinesterase activity of the fresh water snail *Lymnaea acuminata*

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ABSTRACT

The freshwater snail *Lymnaea acuminata* is the intermediate host of *Fasciola gigantica* which cause animal and human fasciolosis, respectively in different part of India. Vector snail *Lymnaea acuminata* is fast breeder and it lays eggs round the year. Temperature is an important abiotic factor which controls the metabolism of snail. High temperature increases fecundity of the snails. A population of glial cells in the central nervous system of the gastropod mollusk *L. acuminata* produces a soluble protein that specifically binds acetylcholine and alters the reproductive capacity of the snail keeping in different temperature in different months of the year Nov-2011- Oct- 2012.

Key words: Fecundity; Temperature; AChE; *Lymnaea*; Caudo-dorsal cells (CDC).

INTRODUCTION

The snail *Lymnaea acuminata* is the vector of the liver flukes *Fasciola hepatica* and *F. gigantica* which cause endemic fasciolosis of cattle in the Northern part of India [1-3]. Incidence of human fasciolosis is also reported in different parts of India [4]. This disease is characterized by abdominal pain, hypereosinophilia and acute pancreatitis [5] Jigyashu and Singh [6] reported that abiotic factors such as temperature play important role in controlling egg laying of snail *L. acuminata*. Vector snail *L. acuminata* is fast breeder and it lays eggs round the year [2, 6]. One of the possible approaches to control the incidence of fasciolosis is to interrupt the life cycle of the parasitic trematodes by eliminating intermediate host. It has also been observed by us that acetyl cholinesterase (AChE) in the nervous tissue of *L. acuminata* is very sensitive parameter influenced by temperature [7].

The nervous system plays an important role in the process of adaptation to different temperatures [8]. Molluscan neurons proved to be a useful model for investigation of the cellular basis of neurophysiological processes, given the potential for identification of individual neurons from one animal to another [9]. They have been used for studies of responses to temperature change for some time. Such studies investigations of the temperature dependence of sodium-potassium permeability ratio [10] and the effect of the sodium pump on the resting potential [11].

This neurosecretory cell [12] innervates the

digestive gland sheet, pericardium, heart, and ganglionic artery [13]. The R15 neuron is important for regulation of osmotic balance [14] and it integrates various aspects of egg laying behavior [15]. Moreover, it has been demonstrated that changes in activity of R15 affect hemolymph concentration of ions and metabolites [16]. As a neuron involved in various homeostatic mechanisms and reproductive behavior, it is a particularly interesting model for studying effects of temperature change on neuronal function.

The aim of present study was to explore the possibility whether temperature of pond water can influence the reproductive capacity as well as AChE activity in the nervous tissue of snail in each month of year Nov-2011 to Oct-2012.

2. MATERIALS AND METHODS

2.1. Test animals

Adult *Lymnaea acuminata* (2.25 ± 0.20 cm in length) were collected from local ponds allowed to acclimatize at 25ºC for 72h in each month of the year Nov-2011 to Oct-2012. The pH of the water was 7.2-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.3 mg/L, 5.2-6.3 mg/L and 102.0-106.0 mg/L, respectively.

2.2. Experimental

The bioassay was performed in clear glass aquaria. Each regimen of 5 L was kept in six aquaria separately, containing 20 snails in each aquarium. The aquaria were covered with the wire netting to prevent the animals for escaping. *Lymaea acuminata* laid their egg in form of elongated gelatinous capsules containing 5-180 eggs on the lower surface of leaves of *Nelumbo nucifera*. After every 24h up to 96h, total number of egg laid by the snails was counted in each aquarium. Simultaneously, temperature of water was measured by digital thermometer in each month of the year Nov-2011to Oct-2012.

2.3. Enzyme Assay

After 24h experiment, the snails were washed with water and the nervous tissue was dissected out from the buccal mass for the measurement of enzyme AChE activity.

2.4. Acetylcholinesterase (AChE)

Acetylcholinesterase activity was measured according to the method of Ellman et al. [17] as modified by Singh and Agarwal [18]. Fifty mg of nervous tissue was homogenized in 1.0 ml of 0.1 M phosphate buffer, pH 8.0, for 5 minute in an ice bath and centrifuged at 1000 g for 30 minute at 4ºC. Supernatant was used as enzyme source. The enzyme activity was measured in a 10 mm path length cuvette using incubation mixture consisting of 0.1 ml of enzyme source, 2.9 ml of 0.1 M phosphate buffer (pH 8.0); 0.1 ml of chromogenic agent DTNB (5,5-dithiobis-2 nitrobenzoate) reagent and 0.2 ml of freshly prepared acetylthiocholine iodide. The change in optical density at 412 nm was recorded for 3 min after every 30 seconds. Enzyme activity was expressed as µ moles 'SH' hydrolysed/ min/ mg protein.

2.5. Statistical Analysis

Results have been expressed as mean ± SE of six replicates The product moment correlation coefficient was applied in between temperature, fecundity and corresponding acetylcholinesterase activity of the snail in each months of the year Nov-2011 to Oct-2012 [19].

3. RESULTS

There was a significant ($p<0.05$) time dependent variation in the temperature, fecundity and AChE activity against *Lymnaea acuminata* in different month of the year Nov-2011 to Oct- 2012. The temperature of water in different month of year was in between 10ºC (January) to 34ºC (June). There was a significant ($p<0.05$) positive correlation in between the fecundity of snail and temperature of water in each month of the year Nov-2011 to Oct-2012. Maximum fecundity was noted in the month of June (1036 eggs/20 snails) and minimum (541 eggs/20 snails) in the month of October, respectively (Table 1).

Table 1. Effect of the temperature on the fecundity of the snail *Lymnaea acuminata*.

Months	Temperature (^{0}C)	Fecundity (24h)
November	24	765±4.8
December	18	588±5.8
January	10	732±4.1
February	21	645±9.6
March	22	584±6.9
April	26	951±4.8
May	28	972±4.8
June	31	1036±5.0
July	32	934±5.7
August	34	826±58
September	31	782±2.5
October	26	541±2.8

Each experiment was replicated six times and the value of temperature and fecundity is the mean of six replicate. Product moment correlation coefficient was applied in between temperature and fecundity of young snail *Lymnaea acuminata*.

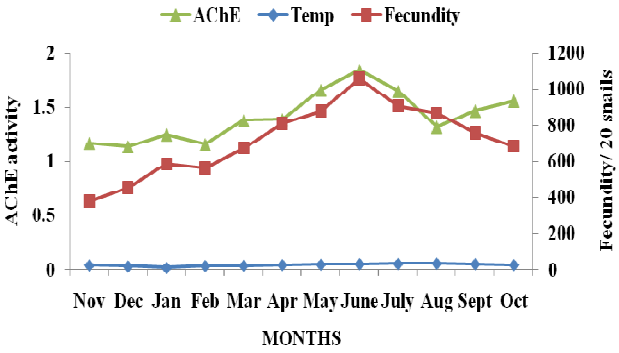

Fig. 1. Effect of the temperature on the fecundity and AChE activity of the snail *Lymnaea acuminata*
Each experiment was replicated six times and the value of temperature, fecundity and AChE are the mean of six replicate. Product moment correlation coefficient was applied in between temperature, fecundity and AChE activity of young snail *Lymnaea acuminata.*

There was a significant ($p<0.05$) positive correlation between fecundity and AChE activity of the snail. Acetylcholinesterase (AChE) activity in nervous tissue of snails was observed in between 1.14-1.84 µ mole SH hydrolysed/min/mg protein. Maximum AChE activity was observed in the month of June (1.84 µ mole SH hydrolysed/min/mg protein) and minimum in December (1.14 µ mole SH hydrolysed/min/mg protein), respectively (Figure 1).

4. DISCUSSION

Result section clearly indicates that temperature of aquatic system play significant role in altering the fecundity as well as AChE activity of snail *L. acuminata*. Studies have shown that there are five major external signals that regulate reproduction in snail: photoperiod, food consumption, temperature, water quality, and parasites [20]. Temperature is an important abiotic factor which controls the metabolism of snail [7]. High temperature increases the fecundity of the snails [6]. It is evident from the present data that increase in temperature in summer months act as enhancer and low temperature act as inhibitor of fecundity as well as AChE activity in the snails *Lymnaea acuminata*.

Bai et al. [21] observed that increase in temperature affect the reproductive rate of the ciliate, *Blepharisma intermedium.* Increase in temperature resulted an increase in organism's reproductive rate. Temperature increases beyond 33ºC the reproductive rate decline [22]. Sudden drops in temperature promote egg mass abortion [23]. A temperature of 25ºC is considered for optimal for oviposition and growth in pomacean snails [24]. Earlier study has shown that decrease in temperature from 20ºC to 8ºC stopped the oviposition of snail *Lymnaea stagnalis*, because of reduction in activities of neurosecretory caudo dorsal cells (CDCs) [25, 26].

This temperature-dependent switch in reproductive activity could occur at one or more components along the reproductive axis. Likely tissues that could be influenced by temperature are the head ganglia, which transmit electrical signals to the bag cells; the bag cells, which synthesize and secrete ELH; and the ovotestis, which responds to ELH by extruding eggs into the hermaphroditic duct. Bag cell neurons from animals maintained at 15°C show significantly lower rates of ELH synthesis than bag cells from animals kept at 20°C [27], potentially affecting the amount of releasable hormone. And treatment of bag cell neurons with α-bag cell peptide (α-BCP, one of several bioactive peptides cleaved from the ELH prohormone and

co-released in response to the after discharge) alters bag cell excitability and levels of cAMP in a temperature-dependent manner [25]. Importantly, activation of the cAMP second-messenger pathway has stimulatory effects on bag-cell membrane excitability [28], ELH synthesis [25], and ELH secretion [29]. Ovulation and egg-laying behaviour are dependent on secretion of the neuropeptide caudodorsal cell hormone [25]. Studies done in *Lymnaea* have identified a number of environmental factors that regulate reproductive function. There is an impressive literature on the details of the cell physiology underlying function of the caudodorsal cells that regulate ovulation and behaviours associated with egg laying [30]. There are six main types of cells/organs known to be involved in regulating egg laying in *Lymnaea*: the endocrine dorsal bodies, the neuroendocrine caudodorsal cells, the lateral lobes, the hermaphroditic gonad, the hermaphroditic duct, and the accessory sex organs (including the albumen gland) [20]. The results also demonstrate that acetylcholine inhibits egg laying, one observation suggests that acetylcholine might also stimulate egg laying. Kim et al. [31] showed that this effect requires the ion channel that serves as the levamisole receptor; however, levamisole receptor mutants are not appreciably defective in egg laying [31, 32] demonstrating that stimulation of this receptor is sufficient but not necessary for egg laying. In fact, nicotinic stimulation of egg laying could have little physiological significance. The fact that reducing all acetylcholine signaling (with *unc-17* or *cha-1* mutations) leads to hyperactive egg laying, and increasing all acetylcholine signaling (with aldicarb or acetylcholinesterase mutations) decreases egg laying, demonstrates that the overriding effect of acetylcholine signaling is to inhibit egg laying [32].

Neurons in the head ganglia (particularly, the cerebral and pleural ganglia) appear to play a central role in the activation of bag cell after discharges [33]. Studies have shown that extract from the atrial gland contains a factor (Peptide B) that when applied to either the cerebral or pleural ganglia activates neural signals to the bag cell neurons, which stimulates the after discharge [34]. Subsequent work investigated the effect of temperature on responsiveness of the head ganglia to atrial-gland extract stimulation of bag cell after discharge [35].

The outcome of this work suggests that temperature has its primary effect on egg-laying behavior as well as through alterations in the responsiveness of command neurons in the head ganglia that ultimately activate the bag cell after discharge. Use of these parameters will be beneficial in snail control programme. It will provide knowledge, when effective snail control method can be applied in the year to control fasciolosis.

AUTHORS' CONTRIBUTION

Both authors contibuted equally to this work, read and approved the final manuscript.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Climate changes mitigate anticipation strategy based on local wisdom - a study of *Tobelo Dalam* tribe (Togutil) in Halmahera Island, Indonesia

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ABSTRACT

The global climate change affects local society. Local people play an important role in conserving forest since it is useful for surrounded environment as water regulator (hydrological), biological variety protection, and climate balance controller. The present study employed qualitative method with participation observation technique in which the researcher involved in the informants' daily activities. The informants were chosen by using *snowball sampling* technique. The data obtained were analysed qualitatively and followed by thematic analysis. The result of the study revealed that the practice of local wisdom in referring to forest protection can be divided into three zones, namely: forbidden forest zone (*Giwengawatoro*), utilized forest zone (*Mialolingiri*), and stream forest zone. On the community utilization aspect, *Tobelo Dalam* tribe (*Togutil*) has a local knowledge related to wood exploitation based on local wisdom through daily live tradition, they are plants for saving water, keeping moist and temperature, fertilize soil, medical treatment, and braid, ritual, aromatic, poison, food, cloth, housing, pesticide, cosmetic, and bio monitoring materials. These local wisdoms were obtained from the ancestors from each generation to conserve the forest through reforestation on a critical land, exploited forest, and stream area. The implementation of the local wisdom in terms of protecting the forest is divided into three zones, they are forbidden forest zone (*Giwengawatoro*), utilized forest zone (*Mialolingiri*), and stream forest zone.

Key words: Mitigation; Climate changes; Local wisdom; *Tobelo Dalam* tribe (*Togutil*).

1. INTRODUCTION

Climate changes affect global warming which also influence the socio-cultural changes. Several social studies found out that the pattern of social relationship is closely related to the pattern of climate changes. If there is no systematic and integrated effort to enhance the tenacity towards the climate changes and rehabilitate the local and global environment condition from now, the impact of climate changes might become worst and in the end it contributes to the difficulty in reaching the continuance development system. A climate changes anticipation in the context of development need an effective climate changed management, which can anticipate the longterm

impact of global climate changes comprehensively. Besides, there also needs cross division approach in the level of national, regional, and local [1]. The global climate changes apparently affect local people. The basic problem is the climate changes do not involve society and its local custom in managing the natural resources. This is the difference between the formal government policy and local people policy and their local wisdom in utilizing natural resources. Related to that condition, it is necessary to reuse the local people ability and authority and their local wisdom to plan, role, control, and manage the natural resources democratically so that it can prosper their own lives. The Local Capacity Development in economics, politics, social, and culture is very important since these aspects are interrelated one another. Thus, the local wisdom of the people should be the core part of the natural resources management and should be promoted widely in supporting the fair policy for the local society and save for the environment [2]

Forest plays important roles for environment, they are water regulator (hydrological), biological variety protection, and climate balance controller. The function of climate balance controller has been known yet the impact has just been realized recently when the climate changes and greenhouse gas emission issues raised. Forest keeps huge amount of carbon substance. About 40% carbon is kept in trees and bushes, while the rest 60% was kept under the soil and roots. Thus, forest is known to be able to sink and release carbon. Besides supporting the biological variety, forest is also needed by human being. Forest and its soil keep a huge amount of carbon, about three hundred billion tons carbon or 40 times emission released to atmosphere. Deforestation and forest degradation contribute to the climate changes in two ways. Firstly, forest over exploitation and fire release carbon dioxide to the atmosphere. Secondly, forest degradation lower the forest area to absorb carbon dioxide. These two aspects are very important for whenever human destroy the rest of forest, human being will be loose in facing the current climate changes phenomena [3]. Climate changes mitigation is an effort to prevent the climate changes through lowering emission and improving the greenhouse gas absorption from several emission source activities [4]. Basically, the forest role in climate changes mitigation is to minimize the emission and enhance the greenhouse gas absorption through photosynthesis process of vegetation. In other words, climate changes mitigation by forest is done through its own ecology function as climate stabilizer. The result of photosynthesis is stored in form of biomass when the vegetation's grow. More carbon dioxide is absorbed when a forest is in a growing stage. It is because the reforestation activity will help the carbon dioxide on the atmosphere. Furthermore, forest can also produce emission from forest conversion or deforestation. Deforestation is a land coverage changes from a place called forest to non-forest. The assumption of this phenomenon is the decreasing forest area which lessens the carbon dioxide absorption potential from the atmosphere.

The importance of mitigation is revealed in Forestry Ministerial Decree No: P.70/Menhut-II/2009 by including Mitigation and Climate Changes Adaptation of Forestry Sector to one of eight policies of forestry development priority in 2009-2014 [5]. The policy regarding to the climate changes mitigation in forestry sector is aimed at an irreversible forest management. The forestry division activity related to the climate changes mitigation is divided into three, they are carbon absorption intensification (reforestation), forest carbon conservation (maintaining the carbon existence in forest from deforestation, forest degradation, and other forest management), and biomass usage optimization to substitute the fossil fuel directly through biomass energy production or indirectly through substituting materials that need fossil fuel. The easiest way to increase the amount of carbon is by planting and conserving trees so that in order to reach the climate changes mitigation target, the carbon absorption is not only happened within nation forest, but also outside forest. Generally, the mitigation process can be supported by adding the number of carbon through trees plantation program. Several activities related to mitigation have been executed in Indonesia, for instance rehabilitation and reforestation by National Reforestation (*Gerakan Penghijauan Nasional-Gerakan Nasional Rehabilitasi Hutan dan Lahan*), One Man One Tree (*Gerakan Penanaman Pohon Satu Orang Satu*), One Billion

Indonesian Trees (*Gerakan Menanam Satu Milyar Pohon*), and Industry Plantation Forest (*Hutan Tanaman Industri*). The before-mentioned activities are able to outburst the land capacity to absorb and keep the emission especially outside forest area or in critical forest.

Regarding the forest degradation which influences the climate changes, one of remote tribes in Halmahera island, namely *Tobelo Dalam* tribe (*Togutil*) practices their local wisdom in preventing the climate changes. This local wisdom is obtained from generation to generation. Local wisdom is norms that are implemented in a certain society. These norms were built in a long period of time which underwent some revolution together with the development of the society and the environment within local system. That process has been being acknowledged in the people's lives and has become their collective knowledge. Therefore, the norms were believed by the local people as a general truth and believed as the guidelines in doing or not doing things. Besides, the norms also bring several dynamic improvements towards ethical lives of the people.

2. MATERIALS AND METHODS

This study employed qualitative method with participation observation technique in which the researcher involved in the informants' daily activities in order to conduct a deeper study related to the local wisdom of the climate changes mitigation strategy of *Tobelo Dalam* tribe (*Togutil*) in Halmahera Island. The present study was conducted from May to August 2014. The study was conducted in Toboino/Tukur-Wukur Wasile, Halmahera North Maluku Province (Fig. 1). The location of the study, Toboino/Tukur-Wukur, was chosen since people *Tobelo Dalam* tribe (*Togutil*) who live in this area were settled and nomaden. The subject of the study was people of *Tobelo Dalam* tribe (*Togutil*) community who have had settled lives and nomaden. In addition, they also possess knowledge and understanding towards climate changes mitigation from their local wisdom.

The authoritative subjects of the study were chosen through *snowball sampling* technique. The instrument of the study was the interview guideline towards the local wisdom knowledge of *Tobelo Dalam* tribe (*Togutil*) community related to climate changes mitigation. The data obtained were analysed by using qualitative analysis followed by thematic analysis.

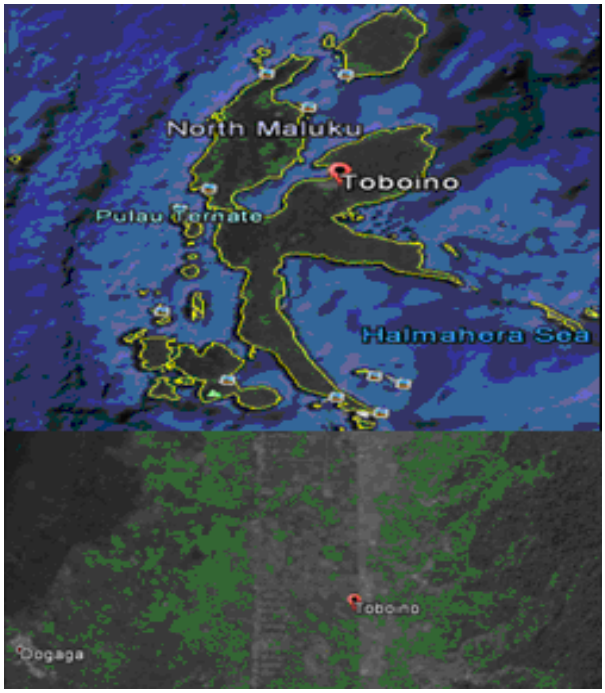

Fig. 1. The study location map (Source: https://earth.google.com).

3. RESULTS

Tobelo Dalam tribe (*Togutil*) possesses several local wisdom which are inherited by the ancestors to every generation, which are called *Ngonyeoko* and *Magilio Ruku* or forbidden forest, utilized forest zone (*Mialolingiri*), and stream forest zone (Fig. 2).

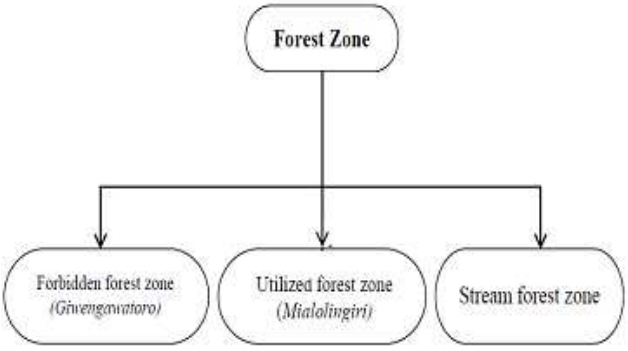

Fig. 2. Forest Zone System Based on *Tobelo Dalam* tribe (*Togutil*).

The community of this tribe live in Wasile, East Halmahera, North Moluccas Province. The people live traditionally and depend much on the forest product. Thus, they respect the nature lives since they have a concept that forest is similar to human being and vegetation own a soul so that they need to be alive. Therefore, human kind needs to take care and make use the natural resource wisely. Vegetation is a living source for every human being, hence, it has to be protected. The ancestors told to the living inheritance (*ngofa-ngofaka*) to not over exploit forest, yet to take it wisely for their own sake. This means that they should not destroy the public area and the forbidden area, in this case forest, which brings advantages to their lives. Hence, conserving the natural source including the vegetation is closely related to their local tradition. The respect towards the ancestors is still influence the people of *Tobelo Dalam* tribe (*Togutil*) lives especially to the natural resource usage.

3.1. Forbidden Forest Zone (taboo) (*Giwengawatoro*)

Forbidden forest zone (taboo) is believed to be a sacred area which is not to be exploited by local people. The local people think believe that there is a mystic energy inside the forest zone. Therefore, local people are not allowed to pass the forest since they might become sick or even die. The only way to enter the forbidden forest zone is done by doing a ritual called *Homaliloa*. The ritual is conducted in regard to ask for well-being guidance by spelling a cast in a local language: *eh nenanga mima doyanga o ka nia-nia ngongaha ka, ne dia donongoho ho uha ni mi tigi-tigi deo uha mi gagawa ma*, which means "We are Thy inheritance, We beg Thee not to disturb us". The forbidden forest area is very large and is considered as a living support area.

3.2. Utilized Forest Zone (*Mialolingiri*)

Utilized forest zone (*Mialolingiri*) is an area used to provide the daily need of *Tobelo Dalam* tribe (*Togutil*). This zone is aimed at several activities such as cultivate, hunt, and collect. The area brings many advantages to the people of *Tobelo Dalam* tribe (*Togutil*) and tends to make the people to use it wisely. The utilized zone functions as the economic source in which it provides humans' basic needs and increases prosperity. The sources usage might lead the society point of view towards the forest material resources, in which forest is exploited merely for the material value. However, this standpoint is not a problem in the forest conservation performance. Most people in *Tobelo Dalam* tribe (*Togutil*) community have settled, yet a few of them are nomadic. This nomadic living makes them acquire knowledge related to the living pattern to survive in a forest. The purpose of the nomadic living is to avoid a consumptive behaviour and modern living style that tend to be materialistic so that they can protect the forest resources to be balance. The authoritative informant in the present study stated that by living nomadic, they can implement nomadic attitudes such as hunt and use the vegetation as they need. This remote community thinks that they are a part of forest and that makes them have a right to protect and use the forest. Regarding the utilization of the forest, this community possesses a local wisdom related to the vegetation usage based on the local wisdom in their daily lives (Fig. 3).

This tradition is carried out for the sake of the forest conservation, that is to minimize the impact of climate changes in the future. If the number of animal in a certain area lessen, the nomadic people move to another area within the same territory. The length of their staying depends on the number of the resources on that area. The result of the hunting activity is used together with the community. Forest is inseparable part of *Tobelo Dalam* tribe (*Togutil*) people lives for forest plays an important role in the ecology system.

3.3. Stream Forest Zone

Stream forest zone is an important area for *Tobelo Dalam* tribe (*Togutil*) people. A near stream forest area functions as stream controller. This tribe generally build houses surround the river since it is considered as an area to do many activities such as fishing and washing. The condition of stream area has become a priority to be protected. The basic aim of the stream

management is to take a sustainable advantage of its resources so that it will not harm the local environment in the future. This remote people take care of the forest by doing reforestation. The reforestation done by *Tobelo Dalam* tribe (*Togutil*) aimed at reducing the erosion through covering the land with bushes. This can be done in several ways like applying planting pattern, thick planting pattern, row planting pattern, and protecting canals. Besides, the reforestation was done to reforest the critical forest in stream area. The main activity of the reforestation was vegetative plantation in the forest to increase the coverage level optimally as well as give advantage to *Tobelo Dalam* tribe (*Togutil*) people, so that there will be a harmony between the forest function and the need of the people. Regarding to the reforestation process done by *Tobelo Dalam* tribe (*Togutil*) community, it is expected that the local, national, and global climate changes can be reduced. The forest development and the management model based on the local wisdom is originated from ecology, economic, and sociocultural aspects in form of supporting area division into several zones. The climate changes mitigation based on local wisdom is done by *Tobelo Dalam* tribe (*Togutil*) community is knowledge within local culture which is done continuously.

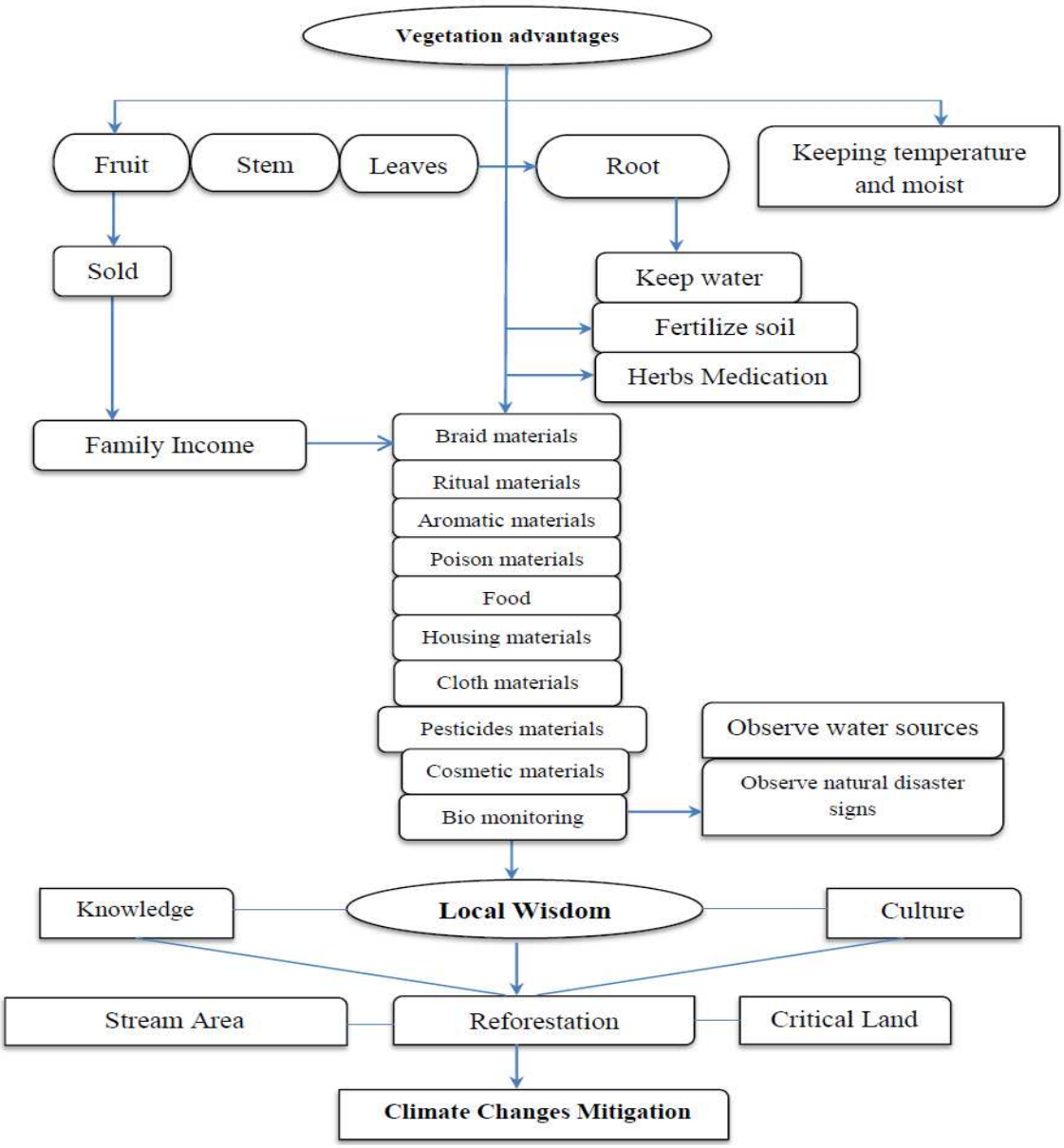

Fig. 3. Vegetation usage based on local wisdom in conserving the forest.

The climate changes mitigation activity based on local wisdom is a local deed which is done by *Tobelo Dalam* tribe (*Togutil*) community to support the climate changes mitigation and adaptation effort and protect the traditional sagacity that can assist the climate changes management and environment damage controller effort in general. The local action that has been done by *Tobelo Dalam* tribe (*Togutil*) community is reforestation in stream area, exploited forest, and critical land. Vegetation plantation strengthens the slide and soil erosion control as well as gives an advantage to soil water conservation effort and critical land treatment. The types of the vegetation used in reforestation depend on the local condition presented in Table 1.

Table 1. The vegetation types for reforestation *Tobelo Dalam* tribe (*Togutil*).

No	Indonesia Name	Scientific name
1	Kenari	*Canarium sp*
2	Mahoni	*Sietenia machrophylla king*
3	Nyatoh	*Palaquium sp*
4	Durian	*Durio zibethinus L*
5	Bintangur	*Calophyllum spp*
6	Benuang	*Octomeler sumatrana*
7	Sengon	*Paraseriantes falcataria*
8	Kayu Bugis	*Koordersiodendron pinnatum*

Besides using that vegetation, *Tobelo Dalam* tribe (*Togutil*) people also reforested the over exploited forest. The reforestation was conducted by grouping some farmers who had been facilitated by East Halmahera government and Natural Resources Conservation Association of North Moluccas. The groups of farmers are called *Maku Tuda* and *Oga Raga*. The role of the farmers are providing seeds, taking care of the seeds, and reforesting the important areas. It is done in sequence to keep the stability of the forest. The utilized forest area is kept for the society needs for they consider the forest as their home.

4. DISCUSSION

4.1. Forbidden Forest Zone (taboo) (Giwenga-watoro)

The more people know the vegetation and the forbidden forest, the more they know the future conservation status [6]. Forbidden forest is an integral part of human being lives all over the world since a long period ago. Zanzibarian respect and protect the forbidden forest for it is a form of protection of the biological variety, unique flower, and fauna. Thus, Zanzibarian use the forbidden forest to eco-tourism only [7]. Forbidden forest influences recent and future society lives and well-being directly. The nature forbidden forest is an important spot in various ecosystems, such as forest, mountain, river, and water resource. The local wisdom of forbidden forest named *ecomistic* is also possessed by *Colo* people in Dawe, Kudus. This concept plays an important role in preventing the environment damage. Self-protection process in form of local wisdom is one way which is done by society to protect the water absorption potential area which is useful for people lives. The environment protection role of the society through *ecomistic*, is done by conserving and caring for the environment. People believe that there is energy beside human that protect the environment as well. The local wisdom of flora can be seen from the local people belief towards *Pakis Haji* (*Cycas rumphii*) and Meranti tree (*Shorea acuminata* Dyer), which have benefit effects. Local wisdom related to local tradition or ritual can be observed from an activity named *sedekah bumi*, which is used as a communication act between human being

and nature [8]. Local people have been doing various efforts to protect forest by implementing customary laws that forbid them to cultivate and take the resources from forbidden forest so that it can be functioned eternally. Dayak Merap and Dayak Punan people also outlaw the society of being cultivate the forest; Some of the animals and vegetation must not be intruded for they are functioned, such as menggris tree (*Koompasia sp.*) on which there are bee combs, rangkong bird (*Buceros sp*) and monkey (*Macaca sp.*) which spread seeds, banyan tree (*Ficus sp.*) which fruit is preferred most by birds, or ulin tree (*Eusideroxylon sp.*) which fruit is preferred by porcupines (*Hystrix sp.*) [9].

4.2. Utilized Forest Zone (Mialolingiri)

The people have a conservation pattern by managing the society forest using the local knowledge based on the experiences and skills. The people have a tradition to protect the ecology balance so well that it does not contribute to the global warming. The reality of life and the society behaviour should inspired others and compensate the carbon exchange value. These people provides a conserved forest for urban people [10]. The effect of traditional management toward the biological variety is well-known widely, and the vegetative regeneration usage becomes a valuable way, in which, basically, locally forest management be able to manipulate the climate [11]. *Enclave* people in Bromo Mount and Sand Sea area are spiritually interrelated to the surrounding forest area. They think that they are part of the nature [12] Rural people possess local knowledge to control the forest related to the reforestation activity, the deforestation decreasing level, and illegal logging since they are involved in protecting the forest, so that the long-lasting and the tranquillity of the forest increase [13].

4.3. Stream Forest Zone

Forest influences climate through several aspects, they are physic, chemistry, and biology process which are affect the planet energy, hydrology cycles, and atmosphere composition. Forest can absorb carbon dioxide that might reduce the effect of climate changes [14]. The mitigation and adaptation strategy implemented by the local people might integrate the local knowledge to the climate changes mitigation and adaptation strategy. The integration of local knowledge can increase the value to develop the extent climate changes mitigation and adaptation strategy which is rich in cultural knowledge [15]. The forest degradation contributes to the climate changes such as extreme weather, long drought, storm, and flood [16]. The increasing greenhouse gas concentration in the atmosphere might cause significant changing in regional climate pattern. This changing affect the variety and the distribution of species for it can influence the ecosystem and the biological variety. The result of the study showed a big biological variety changes which might happen in 2050, that is 32% or 1,990 vegetation species will not exist any longer [17]. A study in Japan showed that a certain vegetation species is nearly extinct due to the global warming; it is generally found in rural area. The main cause is the decreasing habitat because several areas has been re-functioned related to the social economic changes in suburbs [18]. Thus, considering the social and economic factor is an efficient strategy to decrease the greenhouse gas emission [19]. Female role in water conservation, reforestation, food supply, and economic adaptation management is very important in today climate changes phenomena [20]. Moreover, the reforestation strategy has to emphasize on the wider conservation, diversification, and vegetation species distribution. The planting program might use non-local seeds [21]. The local knowledge is a part of scientific approach to understand the climate changes. It is a skill possesses by local people to refer to, to develop the climate changes mitigation concept in the future [22]. The local wisdom prevents spring environment function damage continuously, that is why it should be protected [23].

The environment sagacity contains of the description of the society assumption towards things related to the environmental structure; how the environment works, how the nature react towards human behaviour, and the connections (that should exist) between human being and nature [24]. Natural resources management that integrates forest or woods management and

commodity or short term vegetation plantation, such as agriculture, is important in the climate changes adaptation [25]. Rain forest might contribute to maintaining or improving the forest and vegetation adaptation capacity towards the climate changes and considering the challenge and opportunity to integrate rain forest management. Besides reducing the logging impact to maintain ecosystem integrity, another approach that needs to be done, that is silviculturing a certain plant in purpose of genetic adaptation [26]. One of strategies to anticipate the climate changes is to substitute wood product as fossil fuel [27]. Reforestation is done by selecting the exact plant, bioenergy plant is a kind of carbon mitigation potential plant [28]. Based on the discussion of the study, the land usage or land and forest management change activity can reduce the amount of carbon dioxide on the atmosphere so that it can be included in Kyōto. The activities include reforestation and deforestation (article 3.3 Kyōto Protocol) and agriculture land management rehabilitation (article 3.4).

CONCLUSIONS

The climate changes mitigation anticipation strategy based on the local wisdom of *Tobelo Dalam* tribe (*Togutil*) in Halmahera Island, Indonesia is one of strategies that is applied to protect the forest. The implementation of the local wisdom in terms of protecting the forest is divided into three zones, they are forbidden forest zone (*Giwengawatoro*), utilized forest zone (*Mialolingiri*), and stream forest zone. On the community utilization aspect, *Tobelo Dalam* tribe (*Togutil*) has a local knowledge related to wood exploitation based on local wisdom through daily live tradition, they are plants for saving water, keeping moist and temperature, fertilize soil, medical treatment, and braid, ritual, aromatic, poison, food, cloth, housing, pesticide, cosmetic, and bio monitoring materials. These local wisdoms were obtained from the ancestors from each generation to conserve the forest through reforestation on a critical land, exploited forest, and stream area.

Recommendations

The local wisdoms of *Tobelo Dalam* tribe (*Togutil*) people in Halmahera need to be adapted to the extent development in forestry sector. The central government and the local government need to foster, integrate, and optimize the local knowledge of the local people in managing forest and land as well as anticipating the loosing of local wisdom culture in conserving the forest due to the modern culture influences. It is suggested to the future researches to study the role of people in seaside and small islands in regard to climate changes control based on local wisdom.

AUTHORS' CONTRIBUTION

All authors contibuted equally to this work, read and approved the final manuscript.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Mineralogy and petrographic characteristic of carbonate samples from Kovdor (Kola Peninsula, North Russia)

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ABSTRACT

Revealing in the Kola Peninsula area in Kovdor carbonatites and other alkaline rocks with ultrabazites are samples of the paleozoic hot spot products. They represent a zone of the late devonian intusives, which are common in the Baltic Shield including a Kola Peninsula, Finland, Karelian and NE Poland regions. In these studies were make a microanalyses of rock-forming and accessory minerals using the polarized and SEM-EDS microscopy methods. In these rocks the Nb mineralization was detected.

Key words: Eastern-European Craton (EEC); Carbonatites; Kovdor; Petrology; Mineralogy.

INTRODUCTION

Baltic Shield is exposed part of the East-European Craton (EEC), revealing the northeast of St. Petersburg, and continue performing in the region of Karelia, Finland, Sweden and the Kola Peninsula, disappearing in the north west belong the Caledonian Scandinavian Mountains, located in Norway. The oldest blocks occurring in the Baltic Shield are Kola and Bielamorian. They are exposed mainly in the Kola Peninsula and Karelia. The youngest of magmatic processes in the Baltic Shield is associated with alkaline igneous rocks are represented by a series of intrusions unfolding in Kola Peninsula, Karelia, Finland and in NE part of the Poland. One of these products is an intrusions of Kovdor, Africanda and other smaller. This intrusion is made mostly of carbonatite rock with alkaline and ultrabasic rocks [1, 2].

2. MATERIALS AND METHODS

The collected samples of rocks from the Kovdor were analyzed using an optical polarizing microscope Leica DM2500P and scanning electron microscope Hitachi SU6600 with EDS which are located on the Optical and Electron Microscopy Laboratory in Department of Geology and Lithosphere Protection, UMCS.

3. RESULTS

Kovdor is a irregular carbonate intrusion (Fig. 1), situated in the western part of the Kola Peninsula. It is constructed with ultrabasic products represented by olivinites and pyroxenites which include ore magnetite-apatite-flogopite body [3]. There is a paleozoic intrusion which was

create of the divisions occurring in the older rocks of the Kola Peninsula crystalline basement [4-7].

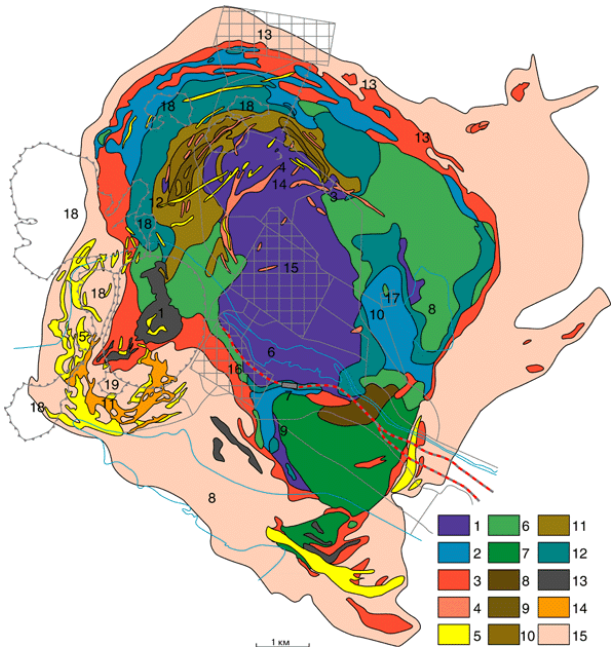

Fig. 1. The geological map of Kovdor [8].
Legend: 1 - olivinite, 2 - melilite-bearing rocks (turjaite, uncompahgrite), 3 - melteigite, ijolite, 4 - felspar-bearing ijolite, nepheline syenite, 5 - carbonatite; 6 - pyroxenite and nepheline-bearing pyroxenite replaced olivinite, 7 - jacupirangite, 8 - biotitite, 9-11 - "Phlogopite complex" (flogopite-diopside-forsterite rocks): 9 -gigantic-grained (pegmatoid) rocks, 10 - coarse-grained rocks, 11 - fine-grained rocks, 12 - montichellite-amphibole, montichellite-phlogopite, diopside-amphibole rocks replaced melilite-bearing rocks, 13 - rocks of the "iron-ore complex" (phoskorite, nelsonite); 14 - apatite-francolite ores; 15 - fenite. Surrounding rocks: gneisses, amphibolites.

Rocks from the Kovdor massif are multi-phase mineralization complex rich in a numerous of minerals such as zircon, francolite, vericulite apatite, diopside, calcite, dolomite, and other. In the vicinity is present a flogopite-magnetite phoscorites and numerous secondary-magmatic rocks [9, 10]. This massive mineralization is rich in trace and rare elements. These phoscorites are rocks with apatite-carbonate-magnetite mineralization. Among of these rocks can be distinguished magnetite-carbonate, magnetite and magnetite-diopside-flogopite phoscorites, olivinites, pyroxenites and metasomatic rocks. The samples of collected rocks type is described on the Table 1.

Table 1. List of analyzed samples of rock from Kovdor.

Sample	Rock type	Sample	Rock type
01KV03	pyroxenite	N4KV03	diopside phoscorite
13KV03	francolite breccia	N8KV03	diopside phoscorite
19KV03	diopside-magnetite-apatite phoscorite	N9KV03	diopside phoscorite
N2KV03	diopside phoscorite	Ph2Kv03	diopside-flogopite phoscorite

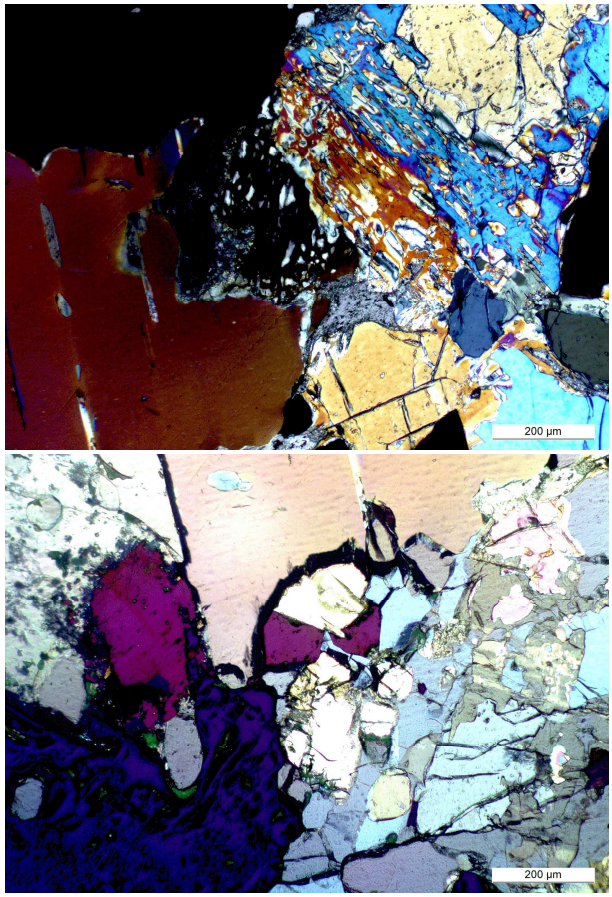

Fig. 2. Microphotographs of the phoscorites in the polarizing microscope (in cross light - up, in reflected light - down).

Phoskorite is a white rocks with black spots associated with the existence of magnetite ore in it. There is a holocrystalic rock of compact, random texture (Fig. 2). In thin section are visible calcite crystals appear automorphic magnetite constructed in a characteristic ditetragonal dipyramids. In the interstitial of these minerals is visible occurring

diopside crystals with apatite and phlogopite sometimes, weathering zone passing in vermiculite.

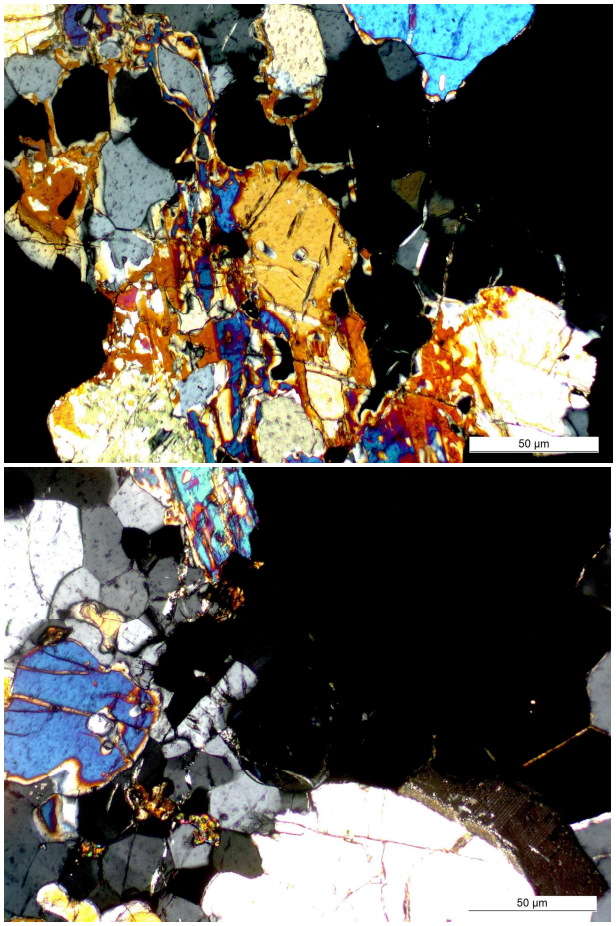

Fig. 3. Microphotographs of the diopside-apatite-magnetite rocks in the polarizing microscope (in cross light).

Diopside-apatite-magnetite rocks, macroscopically gray-green color with visible crystals of apatite, diopside and magnetite. In thin section diopside crystals are visible, with a typical pleochroism in the greenish-straw colors beside which appear repeatedly twinning magnetite crystals. Accompanied by these crystals also apatite (Fig. 3).

Pyroxenites are diopside rock composed almost entirely of these mineral. Macroscopically has a green color, sometimes visible in the large concentrations of magnetite sometimes olivine. In thin section diopside crystals are visible and sometimes accompanying accessory magnetite crystals and olivine (Fig. 4).

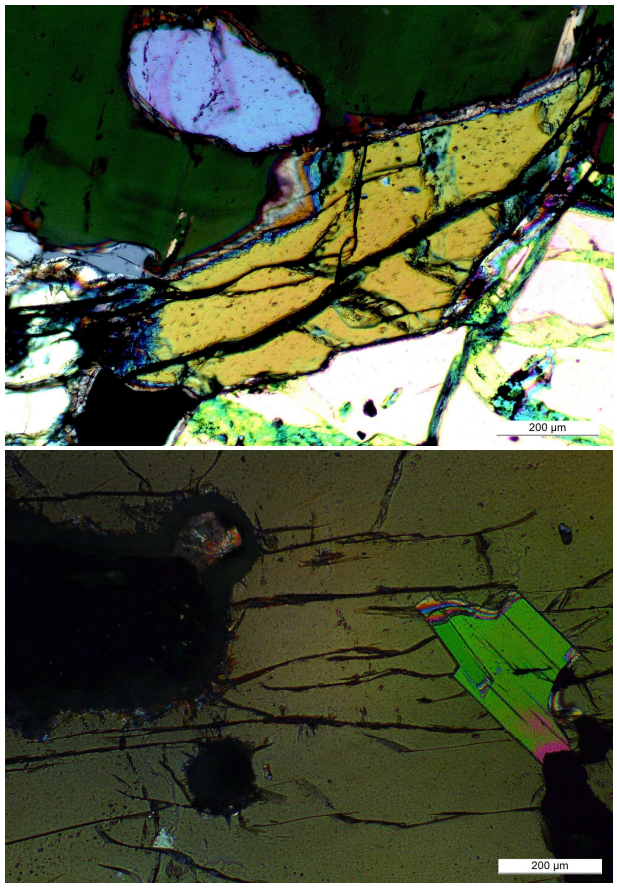

Fig. 4. Microphotographs of the pyroxenites in the polarizing microscope (in cross light - up, in reflected light - down).

Francolite breccia is cream-colored rock, there is usually near foults and weathering zones present. Usually, at first glance, appear pale blue focus francolite like stalactite and sferic formations (Fig. 5). Breccia cement francolie is constructed of apatite crystals with magnetite and sometimes vermiculite. In the thin section francolite other hand, shows a clear focus polycrystalline, composed of spherically arranged microcrystals.

Next francolite are apatite, and magnetite and ambient rock, usually with varying degrees of shredded, most frequently containing carbonates and secondarily modified minerals represented by the vermiculite.

Microanalysis were studied all types of collected rocks. The carbonates calcite dominates with small quantities of magnesium and sodium .In some samples dolomite appears. Depending on the analyzed samples is on the Fig. 6. Next carbonates often appear apatites. The most common varieties are the admixture of calcium or fluorine (Fig. 7).

These apatites appear both in carbonate rocks and other types of rocks (eg. in olivinites). Another issue is francolite breccias, built mainly with phosphates. The results of microprobe analyzes of these rocks are presented in tabular form (Table 2).

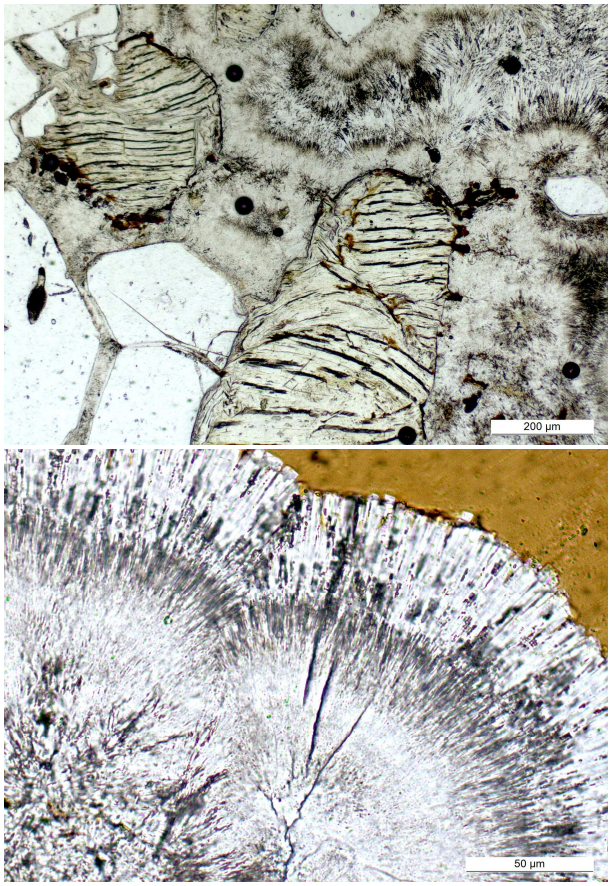

Fig. 5. Microphotographs of the francolite breccia in polarizing microscope (in cross light - up, in reflected light - down).

Their chemical composition does not differ from the studied apatite although in this case is especially interesting form of their occurrence (in the form of spherules). They minerals is forming in the faults zones, where the companion a breccia processes, by binding phase of occurrence rocks. Carbonates and phosphates accompanied niobium inclusions, found mainly in diopside phoscorite and olivinite. It creates a build-up in the range of 15-40% by weight. This is probably the pyrochlore (Fig. 8).

Apart from the above-mentioned minerals, the most commonly encountered rock magnetite. This mineral often associated with small quantities of titanium (Fig. 9).

Olivine occur mainly in olivinites (although also found in phoscorites). Researched olivine is mainly fayalites and hyalosiderite significantly enriched in iron ions (Fig. 10a). Near olivine in the rocks of Kovdor appear feldspars, represented mainly by oligoclase (Fig. 10b). Minerals olivine and feldspar numerous companion a pyroxene. Are found both in the ultrabasic rocks (olivinites, pyroxenites) as well as phoscorites. With clinopyroxenes prevails mostly bronzite (Fig. 11), and hypersthene (found in olivinite, Fig. 12), often coexisting with diopside (Fig. 13). Next diopside also appears augite, especially in the melilite rocks (Fig. 14). Near pyroxene common in these rocks are mica. These are mainly flogopite and sometimes also meets biotite. Some flogopites are vermiculitizing (which is visible even macroscopically).

Table 2. The results of microanalysis (usind SEM-EDS) of francolites) with BSE microphotographs (down).

Sample	O	F	P	Ca
N013Kv03(3)_pt1	38.30	4.20	13.29	41.55
N013Kv03(3)_pt2	39.82	4.77	13.33	39.35
N013Kv03(3)_pt3	37.82	3.94	14.94	40.51
N013Kv03(3)_pt4	38.14	3.61	13.85	42.12
N013Kv03(3)_pt5	35.70	3.37	15.55	41.79
N013Kv03(3)_pt6	37.89	3.59	14.42	42.03
N013Kv03(3)_pt7	41.72	4.78	10.97	31.92
N013Kv03(3)_pt8	38.31	3.89	14.99	40.33
N013Kv03(3)_pt10	37.38	3.69	14.01	38.91
N013Kv03(3)_pt17	38.94	2.50	14.57	40.89

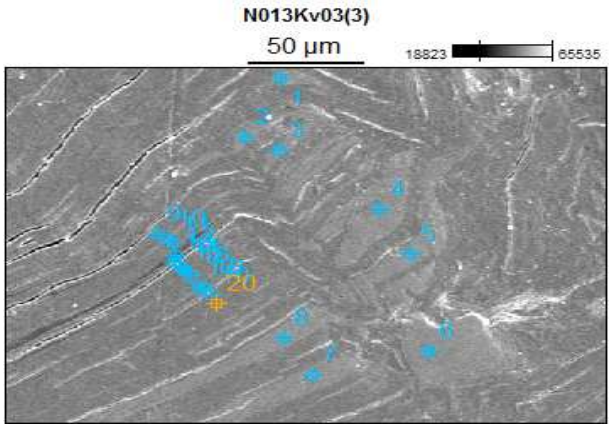

Table 3. Results of the microanalysis of the micas from Kovdor.

Sample	C	O	F	Na	Mg	Si	Al.	K	Ca	Ti	Fe	Al.
N02Kv03(1)_pt13		50,92		2,45	25,32	8,46	12,84		0,00	0,00	0,00	12,84
N02Kv03(1)_pt15	1,97	48,37		2,15	23,43	9,08	15,00		0,00	0,00	0,00	15,00
N02Kv03(1)_pt12	2,21	48,36		0,93	23,44	9,30	15,77		0,00	0,00	0,00	15,77
N02Kv03(1)_pt9	1,99	46,34		2,10	24,68	9,06	15,82		0,00	0,00	0,00	15,82
N02Kv03(1)_pt11	1,31	46,16		3,09	23,99	9,08	16,37	0,00	0,00	0,00	0,00	16,37
N02Kv03(1)_pt17	1,80	48,28		1,08	21,86	10,60	16,38		0,00	0,00	0,00	16,38
N02Kv03(1)_pt16		47,90		2,11	23,68	8,95	17,36		0,00	0,00	0,00	17,36
N02Kv03(1)_pt8	3,18	45,19		0,00	16,06	10,78	18,99	5,80	0,00	0,00	0,00	18,99
N02Kv03(1)_pt10	1,75	45,06		0,00	18,18	9,51	19,82	5,69	0,00	0,00	0,00	19,82
N02Kv03(1)_pt6	2,27	44,41		0,00	15,24	10,31	20,52	7,25	0,00	0,00	0,00	20,52
N02Kv03(1)_pt5		44,73		0,00	16,61	9,28	21,34	8,03	0,00	0,00	0,00	21,34
N02Kv03(1)_pt4	1,89	43,49		0,00	15,65	9,72	21,83	7,42	0,00	0,00	0,00	21,83
N02Kv03(1)_pt7		45,77		0,00	16,10	10,23	22,03	5,86	0,00	0,00	0,00	22,03
N013Kv03(10)_pt13	5,03	33,46		4,82	3,56	21,87	0,00		21,46	0,00	0,00	0,00
N09kv03(1)_pt50	4,70	25,54	1,20	0,41	21,19	14,03	0,00		1,31	0,00	0,00	0,00
N09kv03(1)_pt22	6,55	19,14		0,00	17,89	12,31	0,00		0,00	2,48	3,66	0,00
N09Kv03(5)_pt33	2,91	40,47	0,44	0,00	18,36	13,65	0,00		0,00	4,25	0,00	0,00
N09kv03(2)_pt36	2,37	26,71	2,13	0,00	19,83	12,60	0,00		0,00	4,08	0,00	0,00
N09kv03(1)_pt72	3,11	21,78		0,00	20,97	12,47	0,00		0,00	1,49	0,00	0,00
N09kv03(1)_pt43	3,48	24,23	0,31	0,00	22,46	14,69	0,00	1,20	0,00	0,00	0,00	0,00
N09kv03(1)_pt15	2,82	24,56		0,00	23,51	13,46	0,00		0,00	1,59	0,00	0,00
N04aKv03(1)_pt4	1,98	35,36		0,95	13,89	17,20	5,68	4,87	0,00	0,00	0,00	5,68

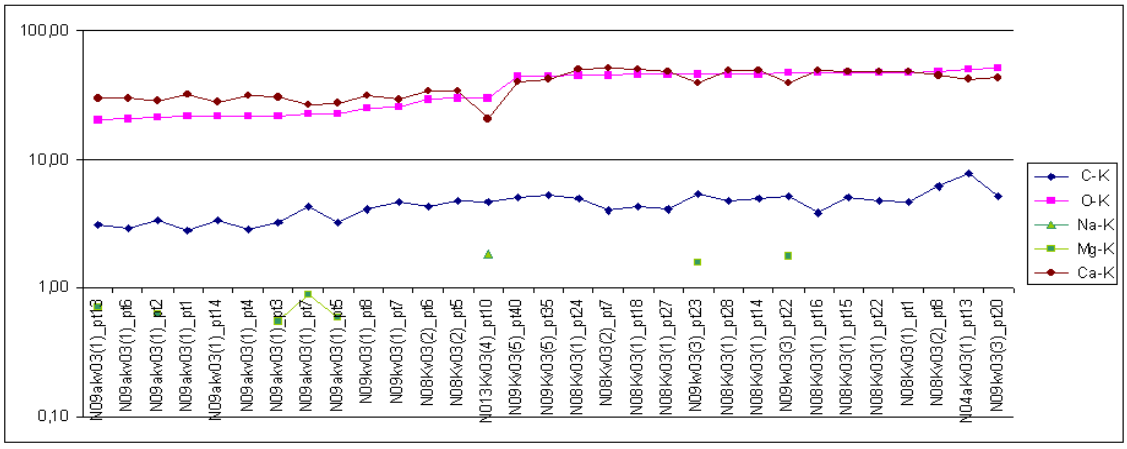

Fig. 6. Characteristics of carbonates.

The results of microanalysis indicate the Table 3 and graph in Fig. 15. Near these described minerals relatively common is zircon (Fig. 16). The Kovdor's compounds of Zr create both silicates and oxides (baddelyite). In some zircons reported a small admixture of Hf.

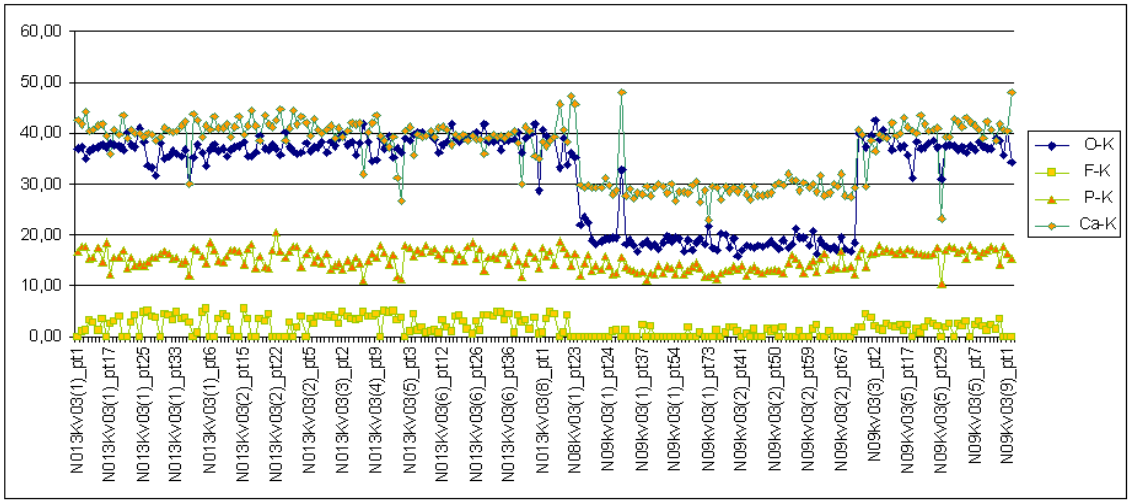

Fig. 7. Characteristics of phosphates.

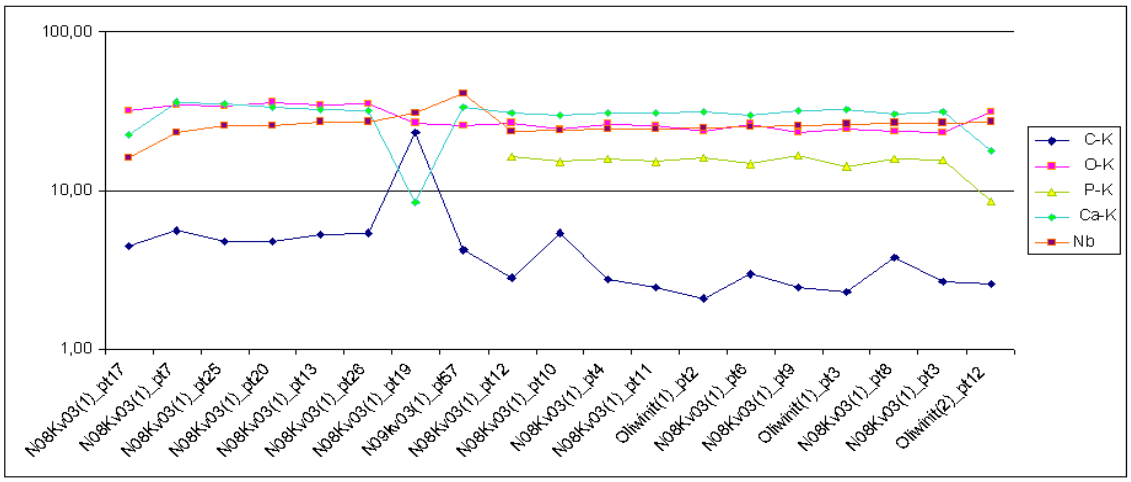

Fig. 8. Characteristics of Nb inclusions in occurrence minerals.

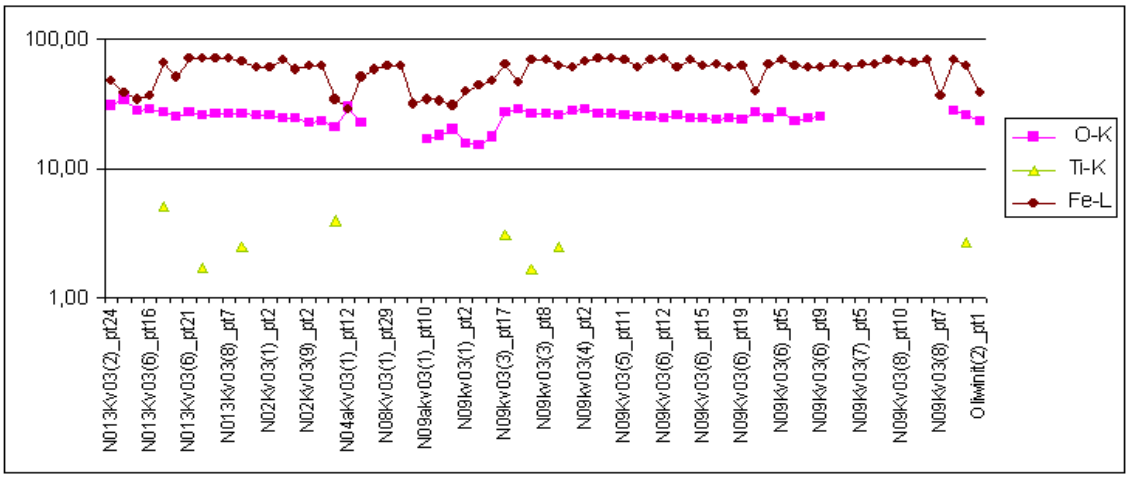

Fig. 9. Characteristics of magnetite.

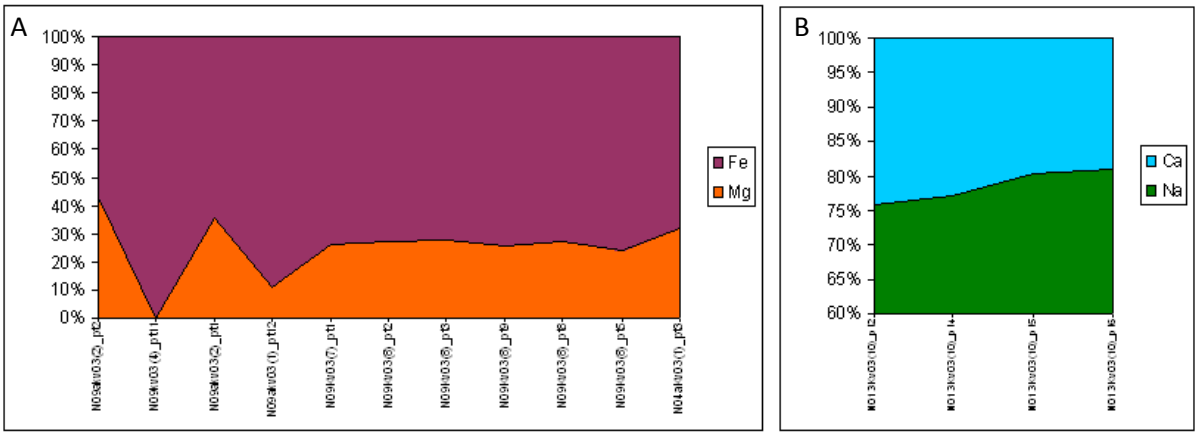

Fig. 10. Characteristics of olivine (A) and plagioclases (B).

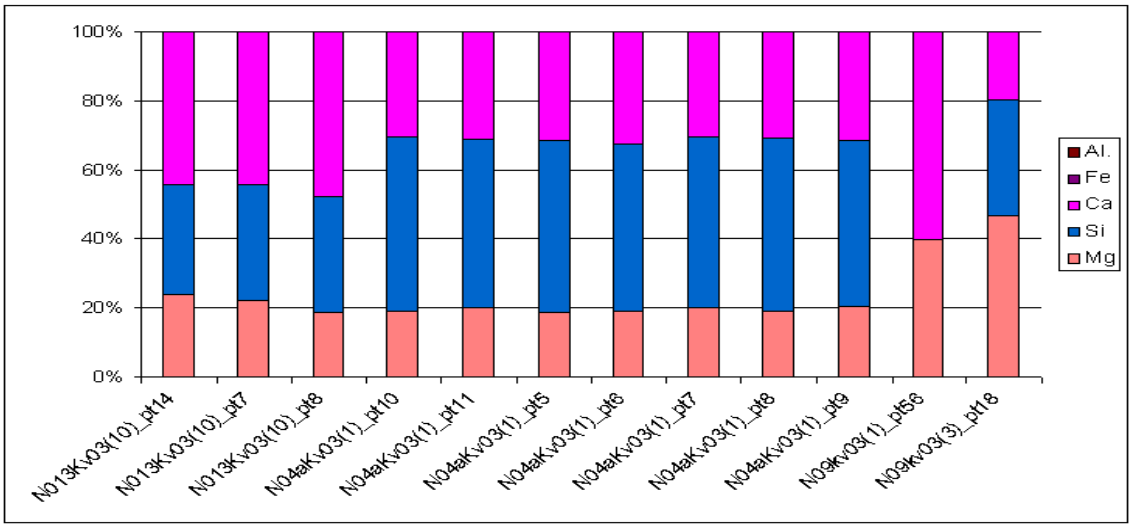

Fig. 11. Characteristics of diopside.

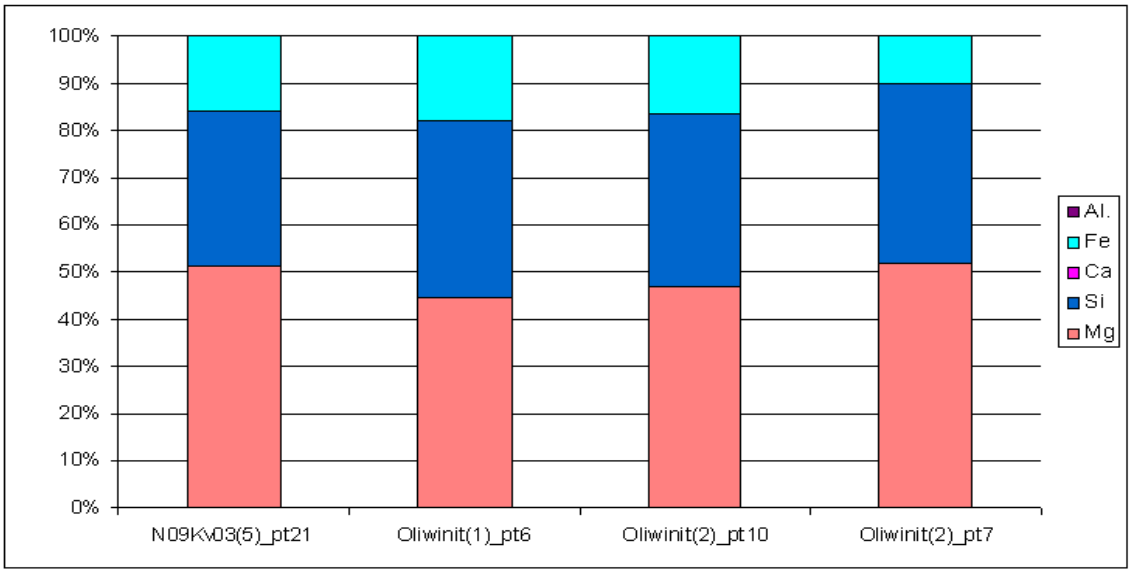

Fig. 12. Characteristics of hyperstene.

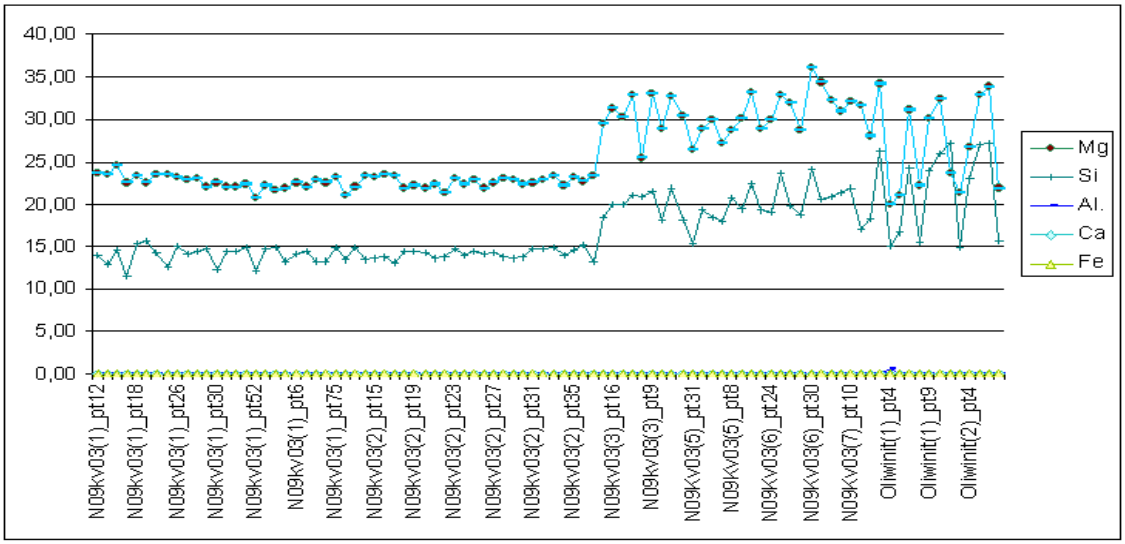

Fig. 13. Characteristics of bronzite.

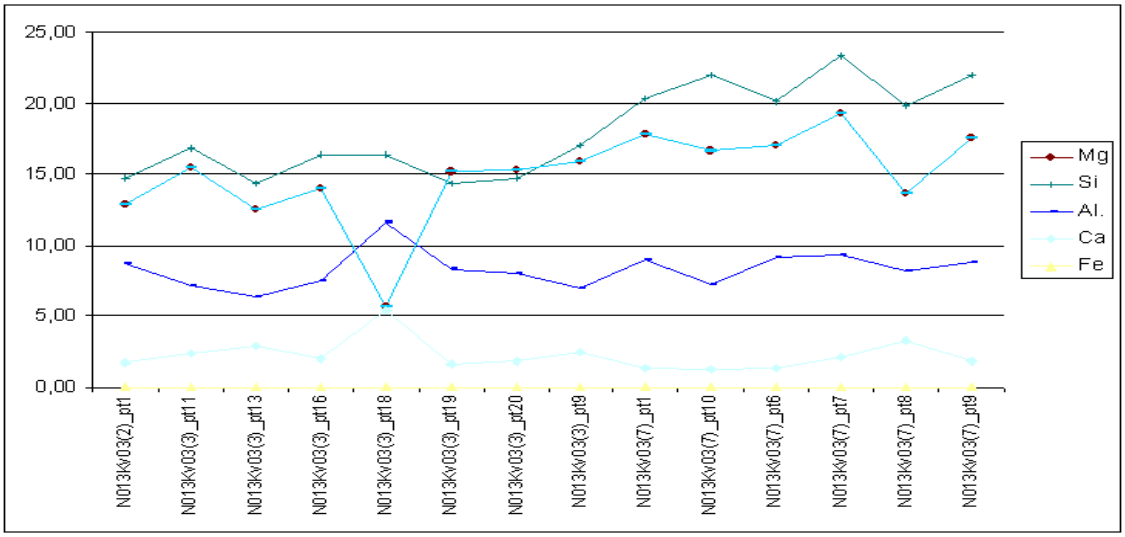

Fig. 14. Characteristics of augite.

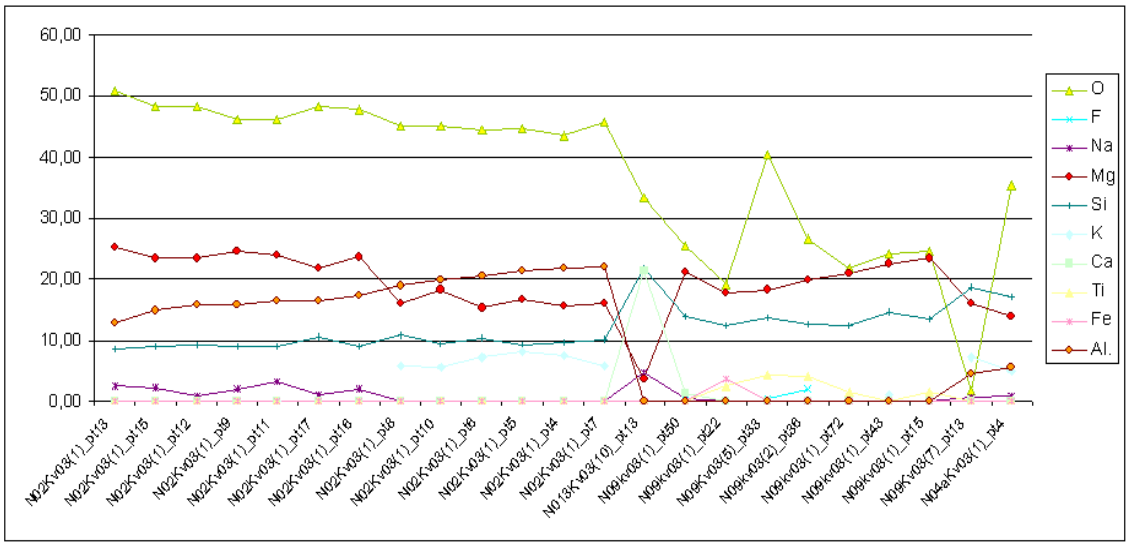

Fig. 15. Characteristics of micas.

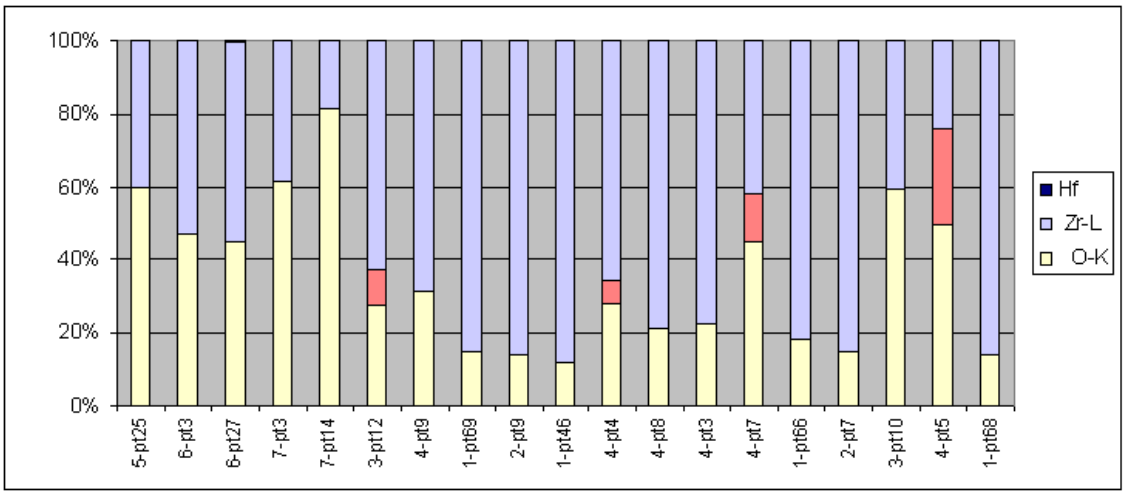

Fig. 16. Characteristics of zircone.

Geology of the Kovdor intrusion is very interesting because of the variety of rocks found in the unveiling. There are both ultrabasic rocks classified as having primary magma injection and associated carbonatites numerous rocks which, in turn, have a large alkaline mineralization with REE elements. Due to the presence of carbonatites and many rare minerals, rocks of Kovdor are unique intrusion located within the Murmansk District. In Khibina and Lovoziero Massif are a large alkaline intrusions but in these masives is present a syenites moustly with a small addition of carbonatites. The Africanda massif has a small surface and different geology. It is present a carbonate veins in Piechenga and Kandalakshan Part of the Lapland Granulite Belt, but these veins have a marginal importance [3].

CONCLUSIONS

Studied rocks samples from Kovdor show a large variety. There are both basic rocks (olivinites, pyroxenites) and carbonatite. Commonly found in the rocks of the presence pyroxenes, ore minerals, apatite. Accessory minerals are flogopite, vermiculite, biotite, zircon like. Of interest are identified impurities of rare elelemnts like Nb mineralization. All of these samples were performed and their mineralization rock intrusions Kovdor makes very interesting from the point of view of the geological structure and mineralogy. Analyzed rock samples are their initial inventory, which will be continued in the near future.

AUTHORS' CONTRIBUTION

Both authors contibuted equally to this work, read and approved the final manuscript.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Bioconcentration of hexavalent chromium in *Cirrhinus mrigala* (Ham 1822): effect on haematological parameters

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ABSTRACT

The study was carried out with intents to measure the relative bioconcentration of chromium (Cr^{6+}) in different vital organs, and to assess the effect of chromium on hematology in *Cirrhinus mrigala.* The fingerlings of *C. mrigala* were exposed to different concentrations of Cr^{6+} (LC_{50} for 96 h was estimated as 52.187 mg L^{-1}) in the form of $K_2Cr_2O_7$. For sub-lethal toxicity test, the fish were exposed to different concentrations of T0 (without chromium), T1 (3.479 mg L^{-1} Cr^{6+}), T2: 5.218 mg L^{-1} Cr^{6+}), and T3 (10.437 mg L^{-1} Cr^{6+}) for 60 days. For estimation of bioaccumulation, the various vital organs (muscle, gills and liver) and haematological parameters, peripheral blood was collected. The chromium-exposed fish showed significantly ($p<0.05$) high chromium concentration in liver (61.91±0.73 µg g^{-1}) followed by gills (16.67±0.08 µg g^{-1}) and muscle (8.97±0.06 µg g^{-1}). The effects of chromium on various haematological parameters showed a significant ($p>0.05$) decreased in the red blood cells (RBC), haemoglobin (Hb) content and packed cell volume (PCV) at the end of 60 days when compared to the control, whereas the white blood cells (WBC) and mean corpuscular volume (MCV) significantly ($p<0.05$) increased in the Cr^{6+} exposed groups. The abnormalities measured during this study are principally imperative because they are associated with bioaccumulation in vital tissues, impaired haematology at sub-lethal concentrations of Cr^{6+} which provides the early diagnostic tools to detect the toxicity of chromium pollution in aquatic environment.

Key words: Chromium; *Cirrhinus mrigala*; Haematology; Bioconcentration.

INTRODUCTION

The chromium (Cr) is well-known to be toxic to living organisms due to their bioaccumulation and non-biodegradable properties. The waste water generated by tanneries is a major source of chromium which contains Cr (VI) ranging from 40-25,000 mg/L. According to Indian standards, the maximum tolerance of total Cr for public water supply is 0.05 mg/L. Chromium (VI) salts have several applications in diverse industries and their indiscriminate introduction into the aquatic ecosystem pose a serious threat to the growth and survival of the aquatic fauna including the fish populations [1]. Chromate ion (CrO_4^{2-}) is the dominant form of hexavalent chromium (Cr VI) in the aqueous solution and can readily cross cellular membranes. The toxicity

of chromium to aquatic life is strongly influenced by the chemical speciation of chromium and water quality [2-3] and considerably varies between and within groups of organisms [4]. Cr (VI) compounds readily penetrate into cell membranes via anion transport systems. Fishes, being at the top of the aquatic food pyramid, may bio concentrate large quantity of certain metals from water [5]. Apart from adsorption on tissue and membrane surface, fish may assimilate metals by ingestion of particulate material or food in water, or ion-exchange of dissolved metals through lipophilic membranes, e.g. the gills [6]. Growing awareness about aquatic pollutants generating potential hazards has stimulated much interest in the use of fish as indicator for monitoring of environmental mutagens, carcinogens and teratogens [7]. Fish have been largely used as bioindicators for environmental pollutants [8-9] and have been used to estimate the influence of environmental pollution due to the sensitivity of their biochemical and hematological parameters under such conditions [10]. Heavy metals accumulate in tissues of fish and may pose a health risk to those who frequently consume them.

The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as heavy metals, biocides, pesticides, industrial effluents, etc. Acute exposure of chromium causes several health disorders to the aquatic life such as effects on the blood parameters (RBC, WBC, hemoglobin, leucocytes, platelets) and functional impairments of vital organs (gills, liver and kidney) [11-13]. Keeping in view the cytotoxicity of chromium and the fact that hematological investigation have proved to be a sensitive tool to detect the effects of chemical compounds within the target organ of fish in the laboratory experiment, the present study focused on chromium-induced hematology of *Cirrhinus mrigala*, one of the most important major carp of India. Hence, the study was carried out with objectives to measure the bioconcentration of chromium in various vital organs and changes in hematological parameters to evaluate the chronic effect of chromium (Cr^{6+}) in *C. mrigala*.

2. MATERIALS AND METHODS

2.1. Experimental fish and acclimatization

The fingerlings of *C. mrigala* (6-8 cm in size with an average weight of 10±2.5 g) were procured from Arey Fish Farm, Goregaon, Maharashtra, India. The fish were acclimatized to laboratory conditions for 45 days in a fibreglass tank of 1000 L capacity under natural photoperiod (12L:12D), with aeration facility. The fish were fed with commercial pelleted fish diet containing 35% protein. The all physico-chemical parameters of water i.e. temperature (25±2°C), pH (7.2±0.4), dissolved oxygen (5.2±0.5 mg L^{-1}) and ammonia (0.01±0.005 mg L^{-1}) were found to be in the optimum range (APHA, 2005).

2.2. Test chemical and determination of 96 h LC_{50}

Analytical grade potassium dichromate ($K_2Cr_20_7$) (Merck, Mumbai, India; 99% purity) was used as the test chemical for determination of medial lethal dose (LC_{50}) of chromium. A daily renewal bioassay system was used to determine LC_{50} value of chromium for C. *mrigala*, following exposure of 96 h according to standard method [14]. Two hundred and ten fish were used to ascertain the range for definitive test. Six test concentrations (100 L each) of narrow range viz. 45, 50, 55, 60, 65 and 70 mg l^{-1} and a control (without chromium) were taken up to find the LC_{50} value of chromium. Ten fish were placed in each of the FRP tank (200 L) and triplicates were maintained for the above treatments including the control. No feeding was done during the experiment. Dead fish were removed from the tank immediately. Death was assumed when the fish were immobile and showed no response when touched with a glass rod. The percentage mortality was recorded at 24, 48, 72, and 96 h interval. Data obtained from the experiment was processed by Probit analysis [15].

2.3. Experimental design

Three hundred and sixty fish were used for conducting bioaccumulation study of Cr^{6+} and its impact on hematological parameters of experi-

mental fish. Three different concentrations of chromium i.e. 1/5th (10.437 mg l^{-1}), 1/10th (5.218 mg l^{-1}), 1/15th (3.479 mg l^{-1}) of 96 h LC_{50} value were taken and a control (without chromium) was also maintained. The treatments were designated as T0 (without chromium), T1 (3.479 mg l^{-1} Cr^{6+}), T2: 5.218 mg l^{-1} Cr^{6+}), and T3 (10.437 mg l^{-1} Cr^{6+}). Three replicates were maintained for each treatment and 30 fish were placed in each of the FRP (fiberglass reinforced plastic) tank of 200 L capacity. Fish were fed with commercial pelleted diet at the rate of 3% of body weight during the experimental period. The whole exposure medium was changed every day in all the treatment including control with a view to maintain the desired concentration of chromium. The water quality parameters were analyzed during the sampling period [14]. During the exposure, mortality and behavior of fish were monitored.

2.4. Estimation of Chromium

Ten gram of fish muscle, gills and liver were dissected out from the fish. In the specimen where 10 g of sample could not be collected owing to small size more than 1 fish (max. 5 fish from each treatment) were taken together to collect sample. The collected sample was cleaned thoroughly with distilled water and kept in a hot air oven at 60^{o}C for 24 hours. The dried samples were ground into fine powder using glass pestle and mortar. About 0.5 g of dried sample was weighed into conical flask for dissolution and to that 10 ml con. HNO_3 and 2 ml con. HCl was added and kept overnight. The flasks were kept on a hot plate for evaporation until brown fumes were completely given out and white fumes were observed. The completely digested samples were allowed to cool to room temperature following which the samples were transferred to plastic bottles and the volume was made up to 25 ml and stored for further analysis. The digested samples were analyzed in triplicate, using Atomic Absorption Spectrophotometer (PerkinElmer AAS, Analyst 800). The blanks and calibration standard solutions were also analyzed in a similar manner as the samples. The heavy metal was analyzed in the fish samples as per standard procedure. The atomic absorption signal was measured as a peak height mode against an analytical curve.

2.5. Haematological studies

Each fish was anesthetized with clove oil (Merck, Germany) at 50 µl L^{-1} of water before collecting blood from fish. Blood was drawn from caudal vein of fish by using 1.0 ml hypodermal syringe and 24 gauge needle, which was rinsed with 2.7% EDTA solution before use. The collected blood was immediately transfered to test tube coated with thin layer of EDTA (as an anticoagulant) and shaked well in order to prevent haemolysis and clotting of blood.

The total leukocyte counts (10^3 mm^{-3}) were determined by taking 20 µl of blood sample mixed with 3980 µl of WBC diluting fluid (Himedia, India) and total erythrocyte counts (10^6 mm^{-3}) were determined by taking 20 µl of blood sample mixed with 3980 µl of RBC diluting fluid (Himedia, India) in a clean vial [16]. The diluted fluids were observed and cells were counted in Neubauer Haemocytometer (Rohem, India). Packed Cell Volume (PCV) was determined by drawing non-dotted blood by capillary action into microhaematocrit tubes. One end of the tube was sealed with synthetic sealant. The sealed tube was centrifuged in a microhaemotocrit centrifuge for five minutes at 5000 × *g*. The PCV was measured using microhaemotocrit reader and expressed as percentage (%). The haemoglobin level of blood was analyzed by the cyanomethemoglobin method using Drabkin's Fluid (Qualigens, India). Blood (20 µl) was mixed with 5 ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer (Thermo Electron, Merck, India) at wavelength of 540 nm. The derived haematological profile of the mean corpuscular volume (MCV; fl), mean corpuscular haemoglobin (MCH; pg) and mean corpuscular haemoglobin concentration (MCHC; %) were calculated according to the equation suggested by Haney et al. [17].

MCV (fl) = Hct (%) × 10 / RBC (million/mm^3)
MCH (pg) = Hgb (gm/dl) × 10 / RBC (million/mm^3)
MCHC (%) = Hgb (gm/dl) × 100 / Hct (%)

2.6. Statistical analysis

The data were statistically analyzed using statistical package SPSS version 16 in which data were subjected to one-way ANOVA and Duncan's multiple range tests (DMRT) were used to determine the significant differences between the means. Comparisons were made at 5% probability level.

3. RESULTS

3.1. Acute toxicity tests

The result of acute toxicity tests, LC_{50} values were found to be 227.08, 55.15, and 52.187 mg L^{-1} for 48, 72, and 96 h, respectively after probit analysis using SPSS software (Probit Method). The fingerlings of *C. mrigala*, exposed to various concentration of the chromium, exhibited clinical symptoms of varying degree depending on the concentrations of the chromium. The test animals showed restlessness, frequent surfacing, and loss of balance and irregular opercula movement, gradually becoming lethargic and in some fish excessive mucous secretion was noticed.

3.2. Bioconcentration of chromium

After 60 days of exposure to different concentrations of chromium, it was found that chromium accumulated in the muscles, gills and liver which was dependent on the duration and concentration and the obtained results are given in Table 1. The Cr^{6+} concentration in gills, liver and muscle varied significantly in all the treatment groups. Significantly high ($P<0.05$) bioaccumulation of Cr^{6+} was found in T3 group of liver tissues, followed by gills and muscle on 30th day of sampling. However, the fish exposed to the treatment T3 could not survive beyond 40 days.

Table 1. Bioconcentration of hexavalent chromium (µg g^{-1}) in different organs of *C. mrigala.*

Treatment	Gills				Liver				Muscle			
Days	15	30	45	60	15	30	45	60	15	30	45	60
T0	0.54^{a} ±0.01	0.63^{a} ±0.02	0.41^{a} ±0.02	0.64^{a} ±0.03	0.65^{a} ±0.06	0.69^{a} ±0.10	0.71^{a} ±0.08	0.61^{a} ±0.18	0.32^{a} ±0.04	0.30^{a} ±0.06	0.28^{a} ±0.08	0.34^{a} ±0.09
T1	4.69^{b} ±0.02	10.52^{b} ±0.1	13.29^{b} ±0.22	15.64^{b} ±0.34	10.61^{b} ±0.20	21.53 ^{b}±0.40	32.65^{b} ±0.29	41.46^{b} ±0.78	3.05^{b} ±0.04	5.50^{b} ±0.16	7.45^{b} ±0.21	8.56^{b} ±0.12
T2	5.64^{c} ±0.03	11.59^{c} ±0.19	13.83^{c} ±0.05	16.67^{c} ±0.08	14.74^{c} ±0.12	22.67^{bc} ±0.17	39.49^{bc} ±0.18	61.91^{c} ±0.73	3.46^{b} ±0.04	6.24^{bc} ±0.03	8.21^{bc} ±0.25	8.97^{b} ±0.06
T3	6.17^{d} ±0.03	13.53^{d} ±0.14	*	*	16.39^{d} ±0.23	25.66^{c} ±0.34	*	*	4.49^{d} ±0.09	7.05^{d} ±0.04	*	*

*The fishes could not survive beyond 40 days of experiment in treatment T3.
Mean values in the column with different superscript differ significantly ($P<0.05$). Data expressed as mean±SE (n=10).

3.3. Hematological studies

At the end of the experiment i.e. after 60 days of exposure, the total erythrocyte counts (TEC) (in 10^{6} mm^{-3}) were found to be significantly ($P<0.05$) low in chromium-exposed groups when compared with control. The TEC values showed decreasing trend in subsequent sampling (Fig. 1). The total leukocyte counts (TLC) (in 10^{3} mm^{-3}) were found to be significantly ($P<0.05$) different in chromium-exposed groups when compared with control on all the sampling days except 45^{th} day. The TLC values were significantly ($P<0.05$) low in the treatment group T1 and T2 whereas high in T3 group on 15^{th} and 30^{th} day of sampling (Fig. 2). However, the TLC values were observed to show increasing trend in subsequent sampling days.

Hb (g dl^{-1}) and PCV (%) values were found to be significantly ($p<0.05$) low in chromium-exposed groups when compared with control on all the sampling days (Fig. 3 and 4). MCV and MCH values significantly ($p<0.05$) differed in all the

chromium-exposed groups 30th day onwards of the sampling when compared to the control. However, MCV values of the treatment groups T1 and T2 did not differ significantly ($p>0.05$) on 15th day of sampling (Fig. 5).

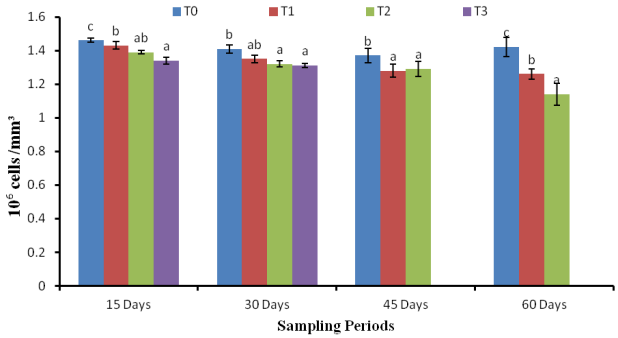

Fig. 1. TEC count of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

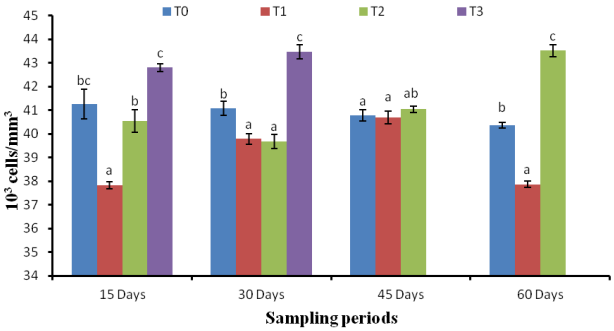

Fig. 2. TLC count of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

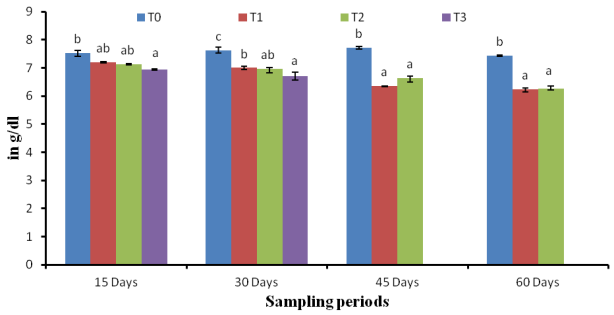

Fig. 3. Haemoglobin (Hb) content of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

Further, MCH values of all the treatment groups were low on all the sampling days when compared with the control (Fig. 6). MCHC values were significantly ($p<0.05$) high in all the treatment groups when compared with control 30th day onwards of sampling (Fig. 7).

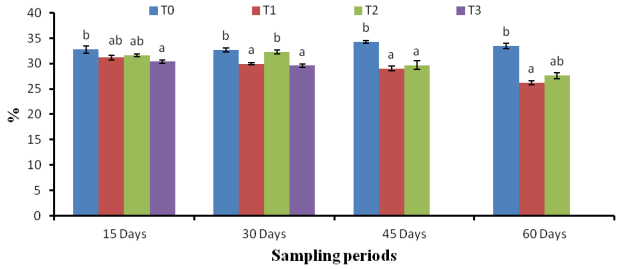

Fig. 4. Packed cell volume (PCV) content of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

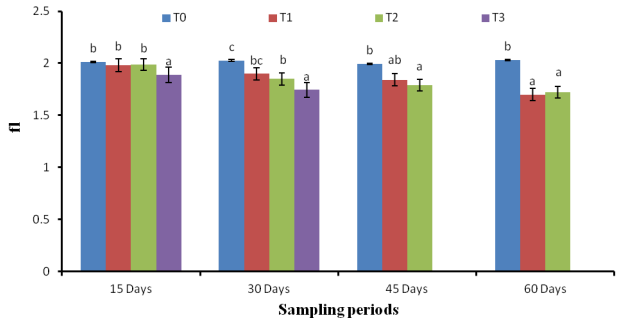

Fig. 5. MCV content of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

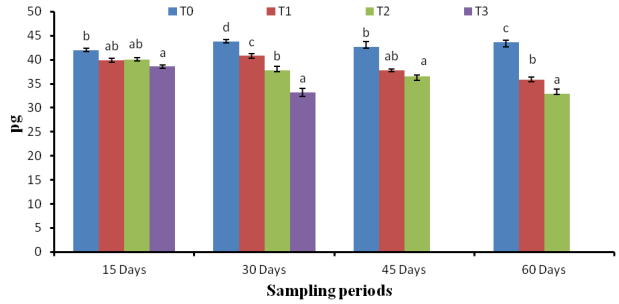

Fig. 6. MCH content of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

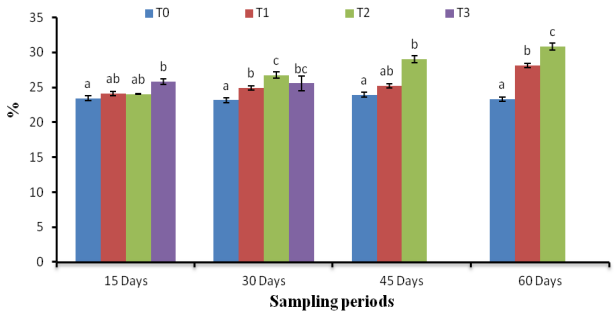

Fig. 7. MCHC content of *C. mrigala* fingerlings exposed to different sub-lethal concentrations of chromium on varied sampling days (values are mean ± SE). Mean values with different superscript on a bar for a parameter is significantly different, ($p<0.05$) n=4.

4. DISCUSSION

The results of the present study revealed that hexavalent chromium exerts effects on haematology of the fish. The 96 h LC_{50} value of chromium for *C. mrigala* (52.182 mg L^{-1}) is high when compared to LC_{50} (41.75 mg L^{-1}) for *Channa punctatus* [1]. Effect of acute doses (LC_{50}) of chromium on fish has been studied in few species, such as *Salmo giardinerri* (140 mg L^{-1}) [16], *Cyprinus carpio* (250 mg L^{-1}) [19], *Oreochromis mossambicus* (200 mg L^{-1}) [20] *Labeo rohita* (142 mg L^{-1}) [21] and *Cyprinus wastoni* (178 mg L^{-1}) [22]. Variations in values may be due to the several factors such as temperature, pH, dissolved oxygen, water hardness and synergism in addition to different fish species [19]. Sanjay et al. [23] reported that toxicity of Cr (VI) in *Channa marulius* was dose and time dependent *i.e.*, with increase in metal concentration and time, the mortality also increased in accordance to present findings where Cr (VI) toxicity was noted to increase positively with concentration and duration. In the present study, hyper-excitation and fast jerking movements were noted in fish before death at higher concentration of chromium. Abnormal behavior (surfacing on water and gulping of air) at higher concentration might be due to manifestation of the disturbances in the physiological mechanism, which is supposed to initiate, maintain and terminate the behavior. These observed behavioral changes in chromium exposed fish are in conformity with the previous report [24]. It also reduced the gaseous diffusion causing less supply of oxygen and causing immediate death of fish [25].

Bio-concentration of chromium in the fish muscle, gills and liver was found to increase depending on the concentration of medium and the exposure time. Giguere et al. [26] reported that heavy metal concentration in fish increased with age of the fish and the exposure time that exerted significant impact on the tolerance limit of fish. Fish liver exhibited greater tendency to accumulate chromium while accumulation of chromium was found to be minimum in fish muscle. However, Avenant-oldewage and Marx [27] reported the general ratio of Cr concentrations between the various organs and tissues being, gills > liver > skin > muscle, and similar results were observed by [28] on *Salmo gairdneri*.

The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants and many of toxicants. Exposure of fish to sub-lethal concentration of chromium for 60 days caused significant variations in blood parameters of *C. mrigala*. The exposure of *C. mrigala* to sub-lethal concentration of chromium significantly decreased Hb, erythrocytes and PCV values leading to anaemia. The anaemia might have led to a fall in the red blood cell count, haemoglobin concentration, and packed cell volume. Heavy metals such as cadmium, chromium, nickel and lead might alter the properties of haemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable, which probably results in cell swelling deformation and damage [29]. Anaemia, under chromium-induced stress, might have been caused due to blood cell injury and disrupted hemoglobin synthesis [30-31]. According to Pamila et al. [32], reduction in haemoglobin content in fish exposed to toxicant could also be due to the inhibitory effect of the toxic substance on the enzyme system responsible for synthesis of haemoglobin. Goel et al. [33] and Kumar et al. [34] have reported similar results with significant reduction of RBC and Hb content of fish exposed to different heavy metals [35, 36], suggesting that heavy metal exposure also decreased the RBC, Hb and HCT due to impaired intestinal absorption of iron. Significance of these changes is well known as reduced oxygen supply results in death of the fish due to heavy metal

pollution. Some of the most common causes of heavy metal toxicity are inflammatory lesions associated with tissue damage, anaemia and neoplasia. The significant changes were recorded in the mean MCV, MCHC and MCH. Similar results have been reported in *Labeo rohita*, exposed to chromium (VI) [13]. Cells released from the spleen, which is an erythropoietic organ would have lowered the MCV values. A similar observation was made in *C. carpio* after cadmium exposure [37]. The significant change in the MCH may be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis [38].

High count of white blood cells indicates damage due to infection of body tissues, severe physical stress, and infection. White blood cell counts were found to have increased following chromium exposure as shown in results. Similar findings were also documented regarding fish exposed to increased level of copper concentration [39-40]. An increase in leucocytes count was also reported in fish, exposed to heavy metals [13, 41].

CONCLUSIONS

The study helps to understand about the level of bio-concentration of chromium in different vital organs of fish, and deviations the haematological profiles from normal in fish under long-term exposure to chromium which provides the early diagnostic tools to detect the toxicity of chromium pollution in aquatic environment. This will help in monitoring and abatement of pollution by employing suitable management practices such as by setting up emission standards for the pollutant, allowable limit of the toxicant, and discharge limits in the aquatic environments. However, further studies are needed to develop more efficient and cost effective tools for early diagnosis of toxic effects of the environmental pollutant.

AUTHORS' CONTRIBUTION

SC: conception and design; AS: development of methodology; SC: acquisition of data; SC and AS: analysis and interpretation of data; SC and AS: writing, review and revision of manuscript; administrative, technical, or material support; AS: study supervision. All authors read and approved the final manuscript.

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TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Morphometric analysis and hydrogeomorphological implication of Paisuni river basin Chitrakoot, Madhya Pradesh, India

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ABSTRACT

The term morphometry is used in several disciplines to mean the measurement and analysis of form characteristics. This study incorporates morphometric linear, aerial and relief aspect of fluvial characteristics of Paisuni river basin. Paisuni river basin covers 571.44 sq km area. Entire drainage network is spread over Sandstones, Limestone and Alluvium with undulating topography. Paisuni river has dendritic drainage pattern. Bifurcation ratio is above 3 which indicate that basin has undulating topography. Mean bifurcation ratio is 3.59 which shows that basin is highly structurally distorted. Relief ratio is 8.8 m/km which shows moderate to high relief of basin. Drainage density & frequency are more and drainage texture is fine which shows that basin is impermeable and having low ground water recharging characters with sparse vegetation. Form factor and circulatory ratio indicates basin shape is elongated. Longitudinal profile shows that basin has high relief in first order drainages and as drainage order increases, relief decreases and finally converts into flat basin at the outlet. Present study is useful to identify the sites for artificial recharging structures to reduce the surface runoff.

Key words: Morphometry; Hydrogeomorphology; Dendritic; Paisuni River.

INTRODUCTION

River basin morphometric elements provide the valuable information for groundwater potential, runoff and geographic characteristics of the drainage basin. The various morphometric properties depend on various aspects like geology, geomorphology, vegetation and climate etc.

Morphometry incorporates quantitative study of the area such as altitude, volume, slope, profile of the land and drainage characteristics [1-3]. It measures and mathematically analyzes the configuration of the earth's surface and the shape and dimension of its landform [1]. Quantitative characteristic of basin morphometry describes the characterization of linear and areal features, gradient of channel network and contributing ground slopes of the drainage. Drainage provides the fundamental principle to understand the initial slope, inequalities in rock hardness, structural control, geolo-

gical and geomorphological history. The morphometric characteristics of various basins have been studied by many scientists using conventional [4-6] and remote sensing and GIS methods [1, 7-13]. Morphological characteristics such as drainage order, drainage density, channel slope, relief, length of overland flow, drainage frequency and other morphological aspects of watershed are important to understand the artificial recharging sites and detail hydrology [14].

2. MATERIALS AND METHODS

Study Area

Paisuni (Mandakini), a holly river of India and its basin falls in between 80° 38' 29"E to 80° 57' 14"E longitude and 24° 50' 51"N to 25° 11' 16"N latitude and covers 571.44 sq km area of MP (Map I). Paisuni River is a perennial river and originates from the central part of Satna district and flows from south west to north east direction and finally joins Yamuna River near Rajpur district UP.

Topographically study area is hilly in southern part and northern part is undulating to plain. Big ravines have area around the both side of Paisuni river channel. Lithologically sandstone, limestone and conglomerate are the main rock types exposed in the area and sandy alluvium [15]. Average annual rainfall of this area is 780 mm. Summer is very hot and maximum temperature rises up to 47^0 C in May. Winter is very cool and minimum temperature goes to 2^0 C in January.

For basin morphometric analysis and hydrogeomorphological implication digital elevation model and toposheets (63 C/16, 63 D/9 & 63 D/13) are used. Drainage network is prepared by toposheets and digital elevation model by ASTER data using geospatial technology.

3. RESULTS AND DISCUSSION

Paisuni River basin is divided into many sub-basins and detailed quantitative analysis of drainage has been done. Detailed morphometric characteristics of Paisuni River basin as linear, areal and relief aspect, is given in the Table 1 to 5, Map 1 and Figs. 1 and 2.

Linear Aspects

In the point of linear aspects we measure some parameters as drainage order and drainage length, mean drainage length, drainage length ratio and bifurcation ratio. The primary step in any drainage basin analysis is order designation, drainage orders and it is based on ranking of drainages [6]. It is noticed that there is a decrease in drainage frequency as the drainage order increases. The drainage length (Lu) has been computed based on the Horton law [16]. Total length of drainage segments is maximum in first order drainages and decreases as the drainage order increases. Mean drainage length is 0.653, 0.694, 1.525, 2.455, 3.378 and 16.082 for different order 1, 2, 3, 4, 5 and 6 respectively.

Drainage length ratio is the ratio between mean lengths of drainages of any two successive orders in the basin [4]. Study shows variation in drainage length ratio between different drainages order i.e. 0.941, 0.455, 0.621, 0.727 and 0.21 for successive order. The lower values of Bifurcation ratio (Rb) are characteristics of the watersheds which have suffered less structural disturbances [6] and drainage pattern has not been distorted [17]. Bifurcation ratio for different order is 3.14, 3.27, 3.22, 7 and 1.34 for successive orders. The mean bifurcation ratio is 3.59 for Paisuni River basin indicates structurally controlled basin (Table 1) [6]. Relief ratio value (Rh) is 8.8 m/km for Paisuni River basin. High values of Rh indicate steep slope and high relief [17].

Areal Aspects

For aerial aspect, different morphometric parameters like drainage density, texture ratio, drainage frequency, form factor, circularity ratio, elongation ratio and length of overland flow are analyzed. Drainage density (1.88 Km^{-1}) is low in Paisuni River basin. The form factor is 0.320, found in Paisuni River basin which indicates less elongated [18]. Circulatory ratio (Rc) is 0.347 and drainage frequency (Fs) is 0.094 found in Paisuni River basin. It is observed that there is maximum frequency in first order drainage and inversely proportional to the drainage order. Drainage texture is 9.176 km^{-1} indicates very fine texture [5]. Drainage density of study area indicates moderate to high runoff and moderate to high impermeabi-

lity [19]. The elongation ratio of Paisuni River basin is 0.641 indicates elongated basin with moderate to high relief (Table 2) [19]. From table 3, it can be seen that lengths of overland flow of Paisuni River basin is 0.265 that indicates lesser infiltration of water which may be due to the presence of compact sandstone and limestone in the area [20, 21].

Table 1. Mean drainage length, Drainage length ratio, Bifurcation ratio and Mean bifurcation ratio of Paisuni river basin.

Sl No	Drainage Order (U)	Total no of drainage (Nu)	Total length of drainages (km) (Lu)	Mean Drainage length (Lū)	Drainage length ratio	Bifurcation ratio (RL)	Mean bifurcation ratio
1.	1	923	603.01	0.653			
2.	2	294	204.00	0.694	0.941	3.14	
3.	3	90	137.26	1.525	0.455	3.27	
4.	4	28	68.74	2.455	0.621	3.22	3.59
5.	5	4	13.51	3.378	0.727	7	
6.	6	1	48.25	48.245	0.21	1.34	
		∑Nu= 1340	∑Lu= 074.77				

Table 2. Drainage density, drainage frequency and drainage texture of Paisuni River basin.

Sl No	Drainage Order (U)	Total no of drainage (Nu)	Total length of drainages (km) (Lu)	Basin Area (Au)	Drainage density Km^{-1} (Dd)	Stram frequency Km^{-2} (Fs)	Drainage Texture Km^{-1} (Rt)
1	1	923	603.01	571.44	1.88	2.348	9.176
2	2	294	204.00				
3	3	90	137.26				
4	4	28	68.74				
5	5	4	13.51				
6	6	1	48.25				
Total		∑Nu= 1340	∑Lu= 1074.77				

Table 3. Elongation ratio, Form factor, Circulatory ratio and Length of over land flow of Paisuni River basin.

Basin Perimeter	Basin length (Lb)	Total length of drainages (km) (Lu)	Basin Area sqkm (Au)	Elongation ratio $Re=2\{\sqrt{(Au/\pi)}\}/Lb$	Form factor $Rf=Au/Lb^2$	Circulatory ratio $Rc=4\pi Au/p2$	Length of Overland Flow $Lg=1/2.Au/\Sigma Lu$
146.25	∑Nu =1342	∑Lu =1074.77	571.44	0.641	0.32	0.3361	0.265

Table 4. Channel gradient, Relief ratio and Ruggedness number of Paisuni River basin.

Elevation of highest point on Basin Perimeter (m)	Elevation of lowest point at the mouth (m)	Maximum Basin Relief (H) (m)	Maximum Basin Length (Lb) (km)	Channel Gradient (m/km)	Relief Ratio (Rh)	Ruggedness Number (HD)
483	111	372	42	8.857	0.0088	0.699

Table 5. Hypsometric data of Paisuni River basin.

Paisuni River Basin			
Hieght (h) meters	**h/H**	**Area (a) sq km**	**a/A**
450	0.93	4.93	0.023
400	0.83	65.94	0.101
350	0.72	139.88	0.258
300	0.62	214.01	0.632
250	0.52	90.03	0.876
200	0.41	44.68	0.991
150	0.31	13.23	1

Relief aspects

The channel gradient is estimated from the contour crossings in the topographical sheet. The overall channel gradient of Paisuni River basin is 8.857 m/km. The Paisuni River basin displays the ruggedness number as 0.694, it indicate, the area is rugged with high relief and low drainage density (Table 4) [19]. Strahler identified three types of landforms, namely, young, mature and monadnock on the basis of hypsometric curve shape. The value of relative area (a/A) always varies from 1.0 at the lowest point in the basin (h/H=0.0) to 0.0 at the highest point in the basin (h/H=1.0) (Table 5).

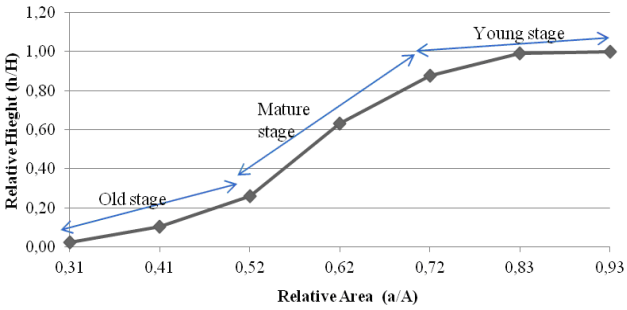


Fig. 1. Hypsometric profile of Paisuni river basin.

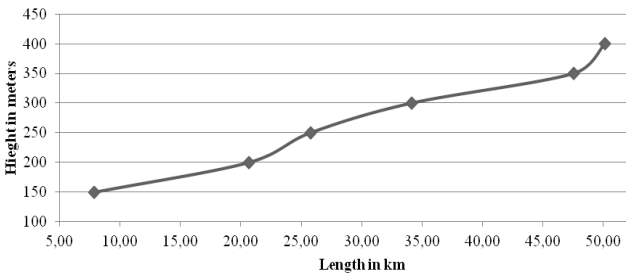
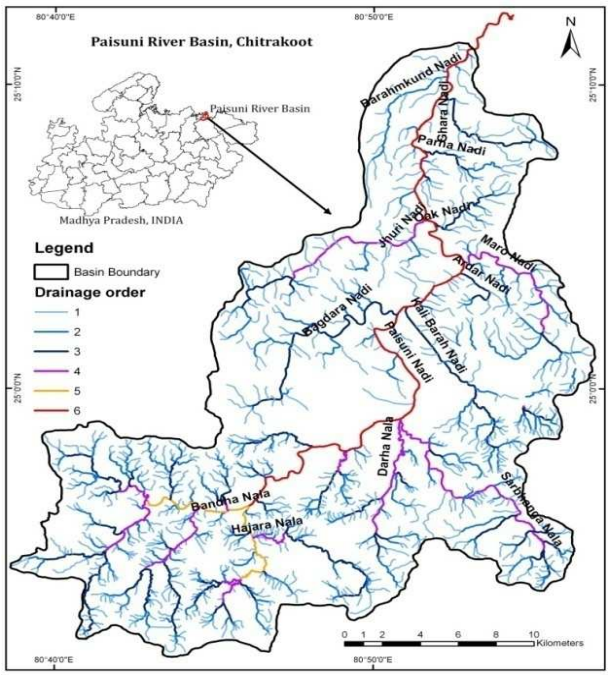


Fig. 2. Longitudinal profile of Paisuni river basin.

Hypsometric curve is showing that younger landform is existing into high relief with lower drainage order. Mature landform is at foot hill with moderate to low relief and older at flat basin (Fig. 1) [22].



Map 1. Location map of study area.

The longitudinal profile is a graph of distance verses elevation. The construction of longitudinal profile provides an interpretation of the surface history as they are the erosional curves and the river course flows from the source to mouth at any stage of evolution [23]. Profile shows that highest and lowest point of basin is 483 and 150 m. Between 400 to 350 m, basin is very steep which spread over 2 km length. Between 350 to 300 m,

basin is about to moderate sloping to plain which spread over 14 km length. Between 300 to 250 m, basin is strongly sloping which spread over 8 km length. Between 250 to 200 m, basin is again moderate steep which spread over 3.5 km length and rest of basin between 200 to 150 m, is gently sloping and spread over 14 km length (Fig. 2).

4. CONCLUSION

The present study deals that Paisuni River basin has dendritic drainage pattern. Morphometric analysis shows that basin is structurally distorted with undulating topography mountainous relief. The runoff is moderate to high. Paisuni River basin is an elongated basin and impermeable for ground water recharging. Hypsometric curve shows that younger landforms are formed on high relief area with lower drainage order. Mature landform is at foot hill with moderate to low relief and older at flat basin. Longitudinal profile shows inverse relation between relief and drainage order and finally converts into flat basin at the outlet of basin.

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AUTHORS' CONTRIBUTION

All authors contibuted equally to this work, read and approved the final manuscript.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

First discovery of melteigite rocks with perovskite from the Khibina Massif

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ABSTRACT

Studied melteigite rocks veins are found mainly in the central part of Khibina on the border of the inter- and outer-zones rocks in the vicinity of deep tectonic faults. These rocks are composed of several stages of mineral associations indicative of changing conditions during the formation of these rocks. The most original ortopiroxenes-olivines are replaced by clinopiroxenes and next going to fenitization with accompanied of alkalines. As a result, around pyroxenes reactionary crown form and structure disintegrating. These rocks indicate the processes of magmatic differentiation in the probable melting zone involving the injection of primary magmas derived from the deep mantle regions, as evidenced by perovskite detected. These rocks ultimately intrude in syenite during tectonic reconstruction of Khibina Massif.

Key words: Melteigites; Perovskite; Minerals characteristic; Microanalysis.

INTRODUCTION

The Khibina Massif is located in the central part of the Kola Peninsula, and is a early-paleozoic high-alkaline intrusion rocks dating back to the age of approx. 350 million years ago [1-3]. Khibina Mountains are composed of alkaline rocks belonging to the group syenites arranged in concentric circles which can be grouped into some sequences. Near the inter- and outer-zones is localized ore bodies with nepheline-titanite mineralization and deep tectonics faults in which is present numerous different type of mineral veins (for example tinguatite, microsyenite) which is visible in numerous places in Khibina. Only melteigite veins are present in the center part of Khibina massif cutting the massive syenites zone and ore bodies [4-6].

2. MATERIALS AND METHODS

During the research, in the study area series of measurements of rocks with photographic documentation were performed. It were made thin plates and then subjected to observation with an optical polarizing microscope Leica DM2500P. These samples were then examined with a scanning electron microscope Hitachi SU6600 with EDS attachment. Studies were carried out in the Department of Geology and the Lithosphere Protection at the Department of Earth Sciences and Spatial, Marie Curie-Skłodowska University (UMCS). Then selected samples were analyzed at the Institute of Chemistry XRD UMCS (Table 1).

Table 1. The type of analysis of the melteigite rocks.

Localization	Sample no.	Type of analysis			
		Planimetric	Microscopic observation	XRD	EDS
Malaya Bielaya Valley	30CH99	Ne-9, Kfs-28, pl-10, dark 52	X	X	X
	17CH99	Ne-0, Kfs-26, pl-16, dark-58	X	X	X
E Pieterlusa slopes exposures	01CH03		X		X
	02CH03		X		X
	05CH03		X		X
	08CH03		X		X
	08aCH02	Ne-3, Kfs-7, Pl-4, Dark69, Ol-7, Ap-10	X	X	X
	09CH02		X		X
	09CH03		X		X
	09aCH02		X		X
	09aCH03		X		X
	11CH03				X
	12CH03				X
	15CH03		X		X
	16CH03				X
	17CH03		X		X
	18CH02	Ne-15, Kfs-15, Pl-9, Dark-55, Ap-6	X	X	X

3. RESULTS

In the massive syenites in central part of the Khibina Mts. near The Malaya Belaya Valley and Ramzaya Pass is present a melteigite veins rocks. Massive syenite, are gray-pink colored rocks composed of plagioclase, aegirine, eudialyte, nepheline and oxides of iron and titanium. They have a coarsely crystalline holocrystalic structure, dense incoherent, texture less directional [7]. Cutting the rock melteigites form dike cores and a width of up to three or even 10 m.

Fig. 1. Eastern Pieterlusa Mt. with numerous mineral veins with melteigite (marked by the arrow).

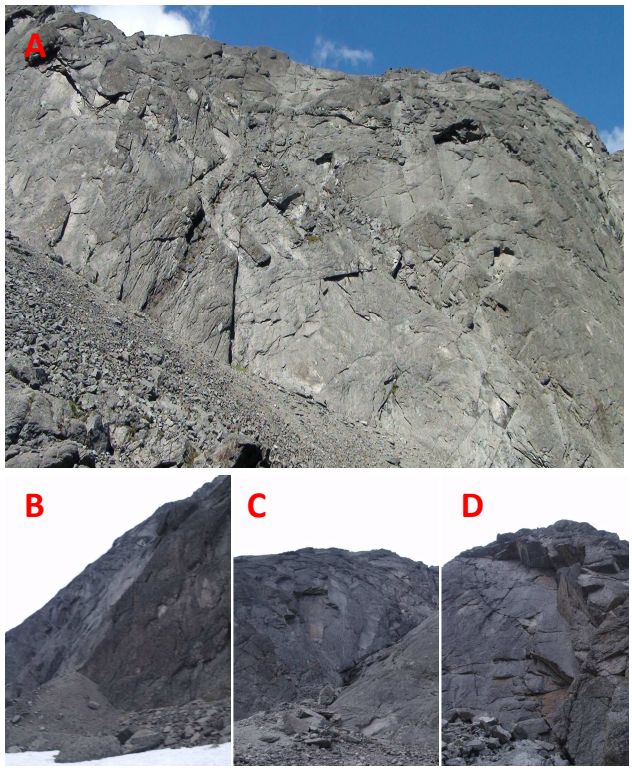

Fig. 2. Approximation of the E. Pieterlusa mountain slopes from the south-west (a), from the South (c), from the south east (near the pass Ramzaya - d) and adjacent Judychvumchorr massif (northern side - b) with visible melteigite veins.

Macroscopically rocks are dark blue-gray with visible in the background rocks pyroxenes, especially in the case of its weathered surface (Figs. 1, 2). Most of these veins occur around the boundary between the inner and outer zones of the rock (Fig. 1). In these rocks especially on fresh fracture appear reactionary olivine surrounded crowns.

In thin section melteigite has a holocrystallic structure, massive, poikilite, porphyre textures. The fenocrystals are augite (to 24% vol., a 57% of whole pyroxenes, Fig. 3a-d), near ther is present arfvedsonite, aenigmatite (to 5% vol., a 2% of femic minerals, Fig. 3a-b). In these rocks are also visible olivine, orthopyroxenes (enstatite) and clinopyroxenes (diopside), which account for about 10% of the volume of the rock (Fig. 3a,e,f). These minerals are usually surrounded by a ringed corrosion (corrosion of their condition varies, met for crystals with a small rim of the end products of the same characteristic). Rims corrosion consist high iron-biotite and phlogopite (Fig. 3e-f). In the background of the rock, there is augite-aegirine and egirine (up to 10% vol., Fig. 3a, d), apatite (up to 10% vol.), nepheline (up to 15%, Fig. 3d), orthoclase (15% vol.) and plagioclase (to 9% by volume, Fig. 3a). Less common rocks developed in the form of pyroxenites of them occurring in random form pyroxenes (augite) suns (up to 79% by volume, Fig. 3d).

A common phenomenon is the passage of augite in aegirine-augite and aegirine (Fig. 3c). In these rocks also noted the chalcopyrite, heikukite and cubanite (Table 2). In other instances, well recognized pyrite and disintegrating zone and chalcopyrite to pyrite sulfates (Table 2). Melteigite veins are more common in the central zones of Khibina, while in the peripheral is present pyroxenites. In these rocks ilmenite and orthopyroxene (augite, diopside) have the structure of disintegrating form (Fig. 4a-d). Pyroxene contained in these rocks has several generations.

The oldest of them along with olivines is clearly corroded, is sometimes even completely disappear. In this group there are orthopyroxenes (enstatite, investigated in studies microprobe) and forsterite. In addition to these phases appear diopside and augite, which also disintegrate. Augite is egirinization, creating continuous transitions that are fenitization. This process is accompanied by a plurality of structures after-spinels disintegration characteristic of demixing of components in the process of recrystallization. As a result, both pyroxene and ilmenite-like structures formed lamelle fractals. These structures are well clear in both: the optical and an electron microscope. Finally the third phase typically associated with alkaline egirine parageneses are augite and aegirine with arfvedsonite, aenigmatite and many emerging nepheline in the spaces between these minerals. In some samples also recognizes the presence of quartz.

Results of microanalysis: 17 selected melteigite samples were performed a 1351 analysis showed some variability of mineral phases. Analyzed minerals are primarily ore phases which mostly consist of ilmenite and titanite mineral admixtures: magnetite, pyrite, chalcopyrite, pentlandite, cubanite and other admixtures such as inclusions containing Pm, W, Sr (Figs. 5a, 6a).

Among dominates albite feldspar and oligoclase, which coexist in paragenesis apatite and nepheline, while there is also a certain amount of basic plagioclase (Fig. 5b). Among the analyzed femic minerals mainly were magnesium olivine forsterite and enstatite accompanied diopside. In subsequent generations are described above clino-pyroxenes. The rocks are dominated phase of augite, which aegirine and titanium augite represents 80% of all femic minerals (Fig. 5E). The corrosion zones are dominated by biotite of phlogopite (Fig. 5F). In melteigites also noticed several fluorites, apatite and lorenzenites whose chemical composition has a number of REE elements admixtures (Fig. 5c, d, 6b).

Studied phosphate phases are mainly chloroapatites and fluoroapatites with numerous additions of various elements (Fig. 6b). Apatites do not differ in chemical composition of the investigated minerals for syenites surrounding these rocks [7].

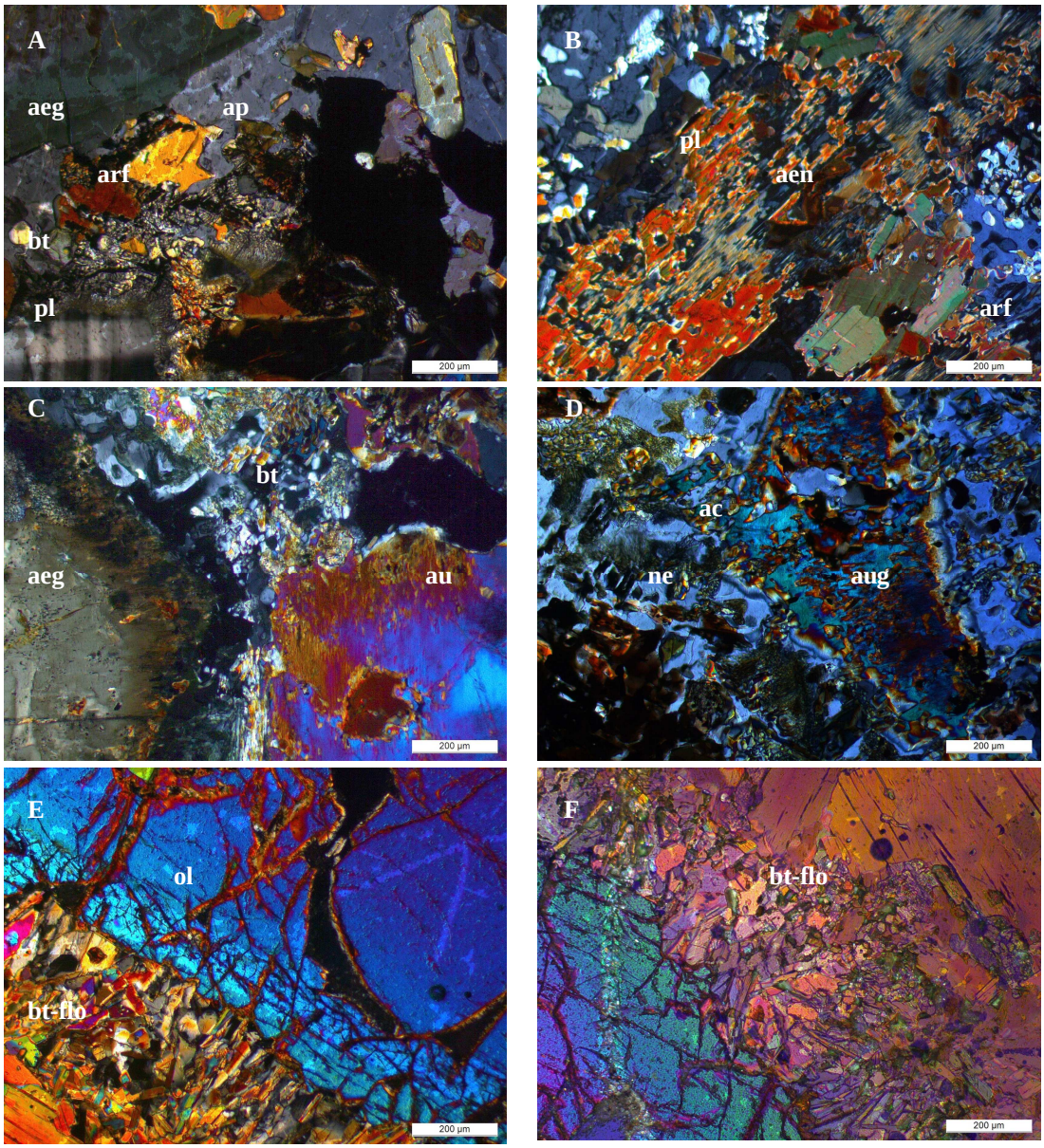

Fig. 3. Microphotographs of typical melteigites: A - aegirine against arfvedsonite, apatite and plagioclase, B - arfvedsonite and aenigmatite with biotite, C - augite border with aegirine unit and mica, D - augite and aegirine against nepheline, E, F - oliwine with ringed corrosive (thin section, crossed pollars, A-D cross E-F reflcted light).

Table 2. Results of microanalyses of some sulphides and sulphates using EDS.

Mineral	Sample	S	Si	S	Ca	V	Fe	Cu	Ni	Mg
chalkopyrite	18ch02(10)_pt13			30,56			26,23	43,22		
chalkopyrite	09ch02(29)_pt1	1,99		22,34			18,77	48,93		
chalkopyrite	09ch02(28)_pt17	5,62		23,87			27,36	34,34		
chalkopyrite	11Melt(3)_pt2	11,22		12,27				48,27		
cubanite	18ch02(2)_pt8			45,93				47,30		
pentlandite	18ch02(4)_pt4	23,57		25,46		3,39			44,91	
pentlandite	09ch02(22)_pt7	6,19		22,49			36,03		28,61	
gipsum	05Melt(3)_pt1	43,21		11,51	8,08					5,07
gipsum	05Melt(4)_pt1	36,48		37,28	6,28					2,15
quartz	11CH3	79,13	20,87							

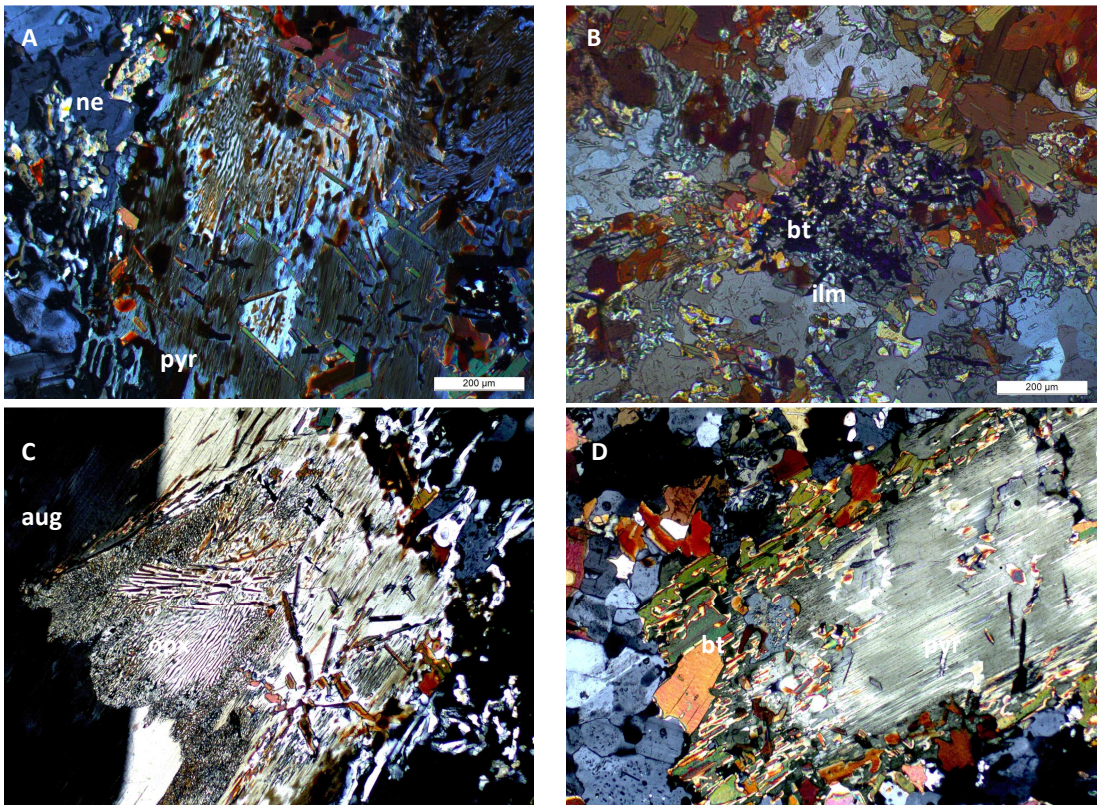

Fig. 4. Microphotographs of the disintegration structures: A - pyroxene against nepheline, B - structure decays ilmenite, C - admixing phases in the pyroxene, D - corona reaction around the pyroxene (polarysed microscopy, crossed pollars, A, C, D - cross, B - reflected light).

4. DISCUSSION

Investigated melteigites are key to understanding the complex construction of the Khibina massif, are in fact one of the last compositions formed the massif though they may contain ingredients of the original magma. Co-occurring mineral paragenesis have a large evolution of what has passed in which the material formed melteigites. The most original of them, made of pyroxene and olivine with spinels probably had ultrabazite character and was able to come from deep in the earth, perhaps even from the mantle, as Khibina intrusion like many others on the Kola Paleozoic intrusion has a close interaction with the hot spot [8, 9]. For this hypothesis may indicate the fact that it was found by microanalysis likely existence perovskite in these rocks as evidenced in tab 3. It is possible that the disintegrating structure found in pyroxenes and ore minerals may also indicate a residual magma as the original, which was pressed into the rock splits Khibina already a supercooled solution.

Table 3. Results of the microanalysis of perovskites.

Phase	Sample	O	Ca	Ti
perovskite	*09ach02(3)_pt2*	42,92	7,81	41,57
perovskite	*09ach02(3)_pt3*	39,98	7,34	35,94
perovskite	*09ach02(6)_pt10*	41,23	12,12	40,17
perovskite	*09ach02(6)_pt12*	42,91	12,03	39,38
perovskite	*09ach02(6)_pt13*	39,63	11,00	34,46
perovskite	*09ach02(6)_pt17*	39,09	9,97	33,51
perovskite	*09ach02(6)_pt18*	38,02	10,82	34,96
perovskite	*09ach02(6)_pt19*	38,97	9,74	36,03
perovskite	*09ach02(6)_pt21*	50,96	12,36	15,73
perovskite	*09ach02(6)_pt26*	39,83	8,52	33,72
perovskite	*09ch02(25)_pt17*	32,82	7,43	16,37

Further assemblage is already associated with the modification of the original chemical composition of the magma due to its contact with alkali fluids. This is evidenced by both the primary phase corrosion and the emergence of the phase characteristic of the syenite rocks like aegirine, nepheline, apatite and visible fenitization of pyroxene (augite). Example preserved fenitization and crowns reaction shows a gradual change environ-

ment and mixing alkaline components in not fully crystallized solution, which must have occurred during the migration and injection. Contact these veins and dykes from the rocks of the environment is usually sharp which could mean that it is likely these changes occurred much earlier, even before they were at this place. Probably also already occurred corrosion and secondary changes in the early stages as the only mineral that could be explained by the fact that the temperature of the rock core intruding could not much affect the surrounding rocks. Perhaps it is a sign of stratification (layered) is in the focus of magmatic intrusion and an example of small injections of residual primary magmas that have been modified in an alkaline environment. When tectonic layers were partially differentiating cumulates thrown syenite rocks, where there was a re-response due to changes in the chemistry and corrosion processes. He had to be, but this process before the final formation of Khibina because this process has contributed to both corrosion and decay in the scales of the original components and pressed them into tectonic faults Khibina as evidenced by the occurrence of these rocks form.

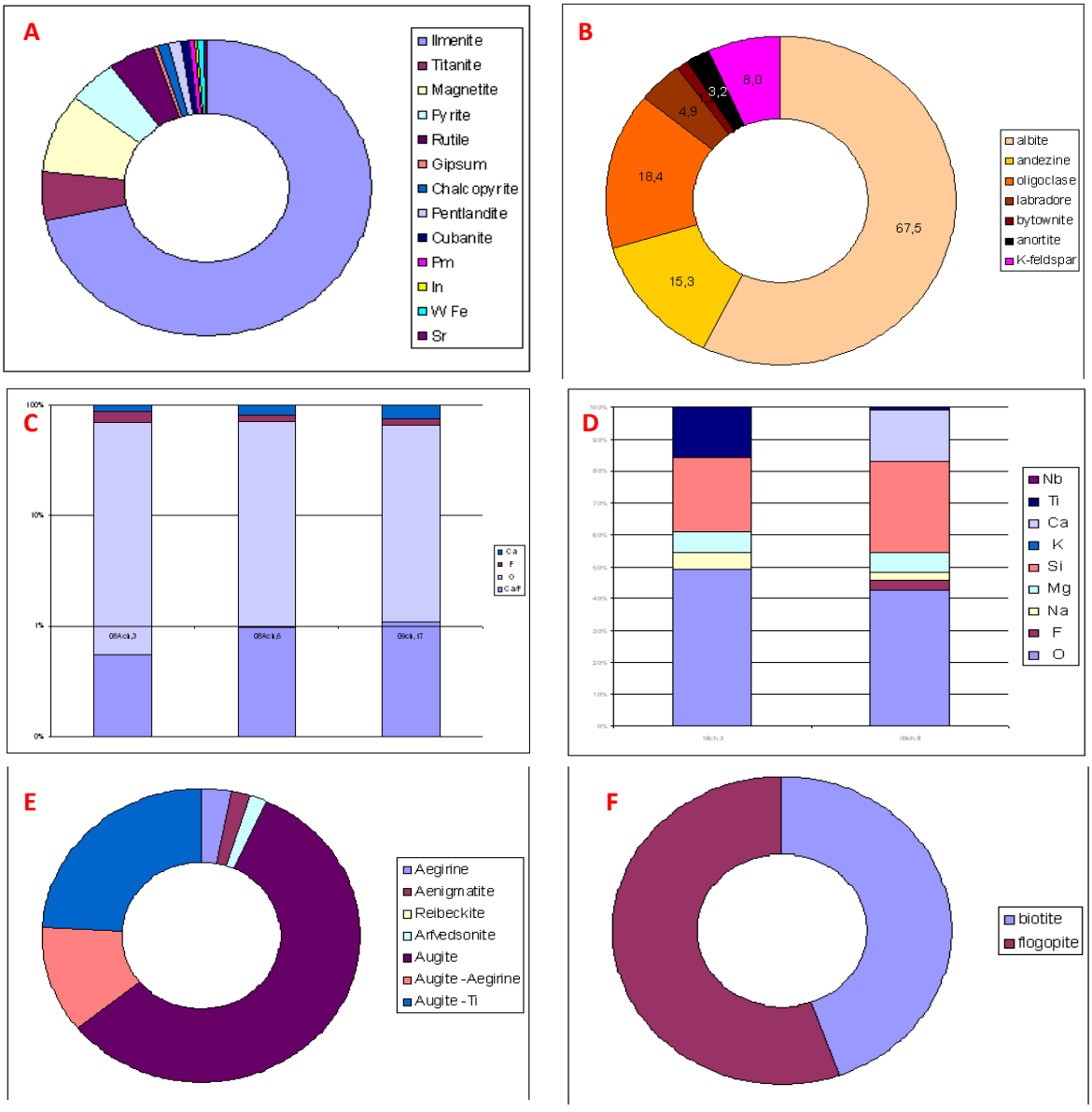

Fig. 5. Diagrams of the results of microanalyses: A – type of ore minerals, B – feldspars, C – characteristic of fluorites, D – lorenzenites, E - characteristic of femic minerals, and F – micas.

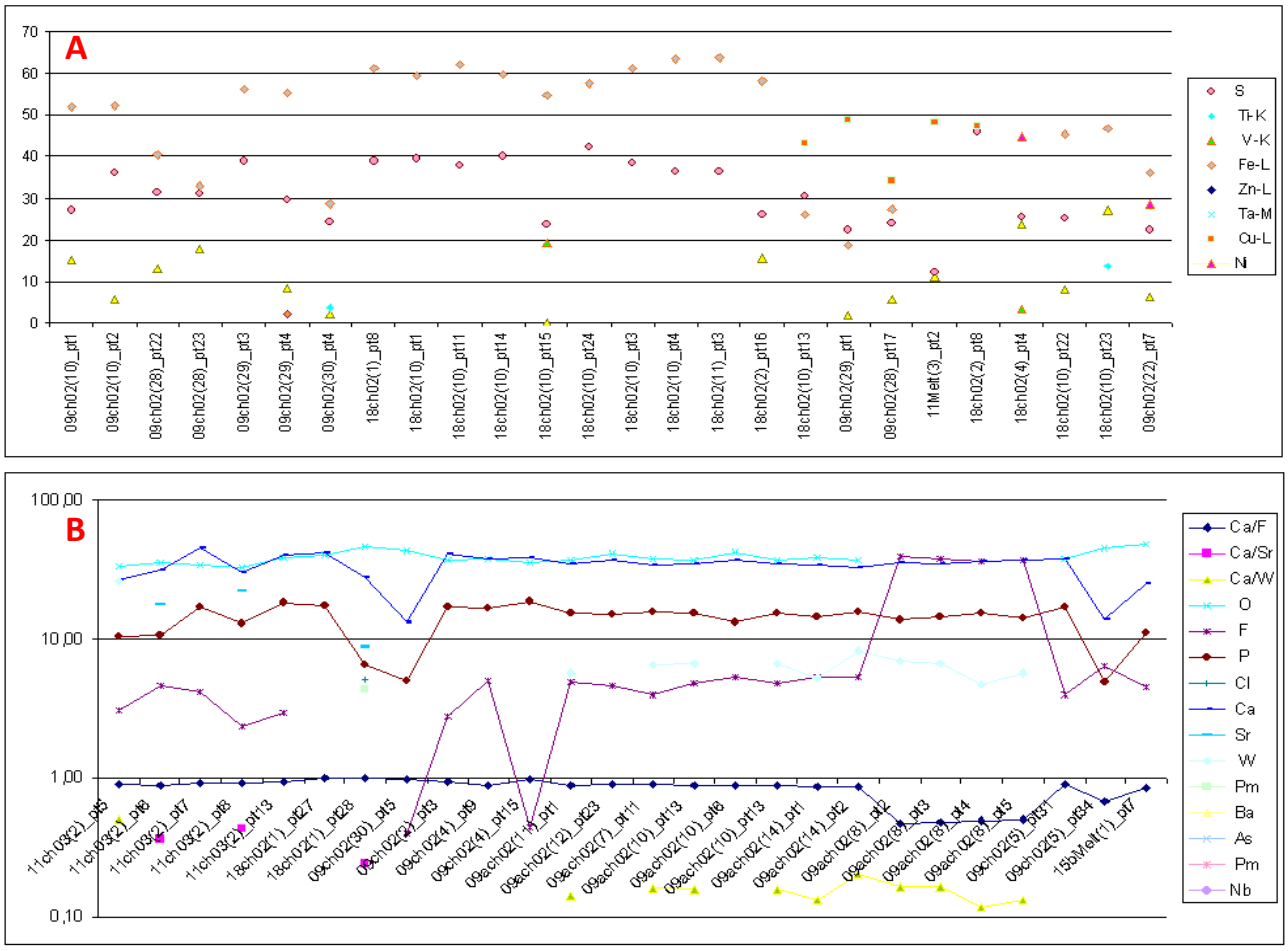

Fig. 6. Diagrams of the detailed analysis of sulphides (A) and apatites (B).

5. CONCLUSIONS

Studied melteigites are special type of rocks. Taking a deep tectonic faults are associated Khibina tectogenesis scale of the intrusion. They are also the key to understanding the origin and relationships of deep fluids derived from the mantle of the Earth, as evidenced by perovskite mineralization and ol-opx association. Further processes leading to pyroxene corrosion and fenititization, alkali rocks accompanied are evidence to the stirring up of these components is likely to penetration of these rocks in the breakaway zone, where the uprising had to be the disintegration of structures and crystallization of alkaline minerals.

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

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ORIGINAL ARTICLE

Microanalysis of alkaline rocks from the Khibina Massif using SEM-EDS methods

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ABSTRACT

Khibina Massif is a central intrusion of the alkaline rocks. There are different types of syenites, urtites trachites and numerous ore bodies. These products arranged concentrically are slightly vergence in the east. Syenite rocks are constructed by plagioclase, ortoclase, aegirine, augite, and other pyroxenes and often accompanied by apatite, nepheline, eudialites and other phases. Interesting are many common ore minerals such as ilmenite, titanite, sulfides. Relatively common in these minerals are numerous impurities and inclusions. There are inclusions of such elements as Sr, Nb, lanthanide and even uranium compounds.

Key words: Khibina massif; Alkaline rocks; Syenites; Pyroxenes; Inclusion of REE elements.

INTRODUCTION

The Khibina Massif is localized in the central part of the Kola Peninsula and there is an early-paleozoic high-alkaline intrusion, which have a 350 Ma [1-4]. Khibina is surrounded Monchegorsk massif from the west, rock of the Kola series from the north, Imandra-Varzuga belt from the south. Khibiny Mountains are composed of alkaline rocks belonging to the group syenites which have concentric forms and 4 sequences: the first one is the outer circle of rocks made up of massive khibinites, alkaline-feldspar syenites, second internal intrusion sequence is also constructed with similarly rocks with massive structure with visible sometimes pegmatites. Between these rocks is present ore zone (third sequence), composed mainly of titanite-nepheline rock and very rich in dark minerals - kakortokites [5, 6]. All of these pieces are cut by numerous veins of a somewhat later (fourth sequence) mainly composed of tinguaites, trachytes and melteigites [2]. These veins of the body which are sometimes up to 3 m in width are accompanied by numerous faults that are associated with the construction of the central alkaline intrusion [7-9].

2. MATERIALS AND METHODS

The collected samples of rocks from the Khibina were analyzed using an optical polarizing microscope Leica DM2500P and scanning electron microscope Hitachi SU6600 with EDS which are located in Department of Geology and Lithosphere Protection, UMCS. It was performed 4779 analyzes from 444 places. In some samples of rocks was made a XRD and IR analysis in Geology, Geophysics and Environment Protection Faculty, AGH Science and Technology University in Cracow.

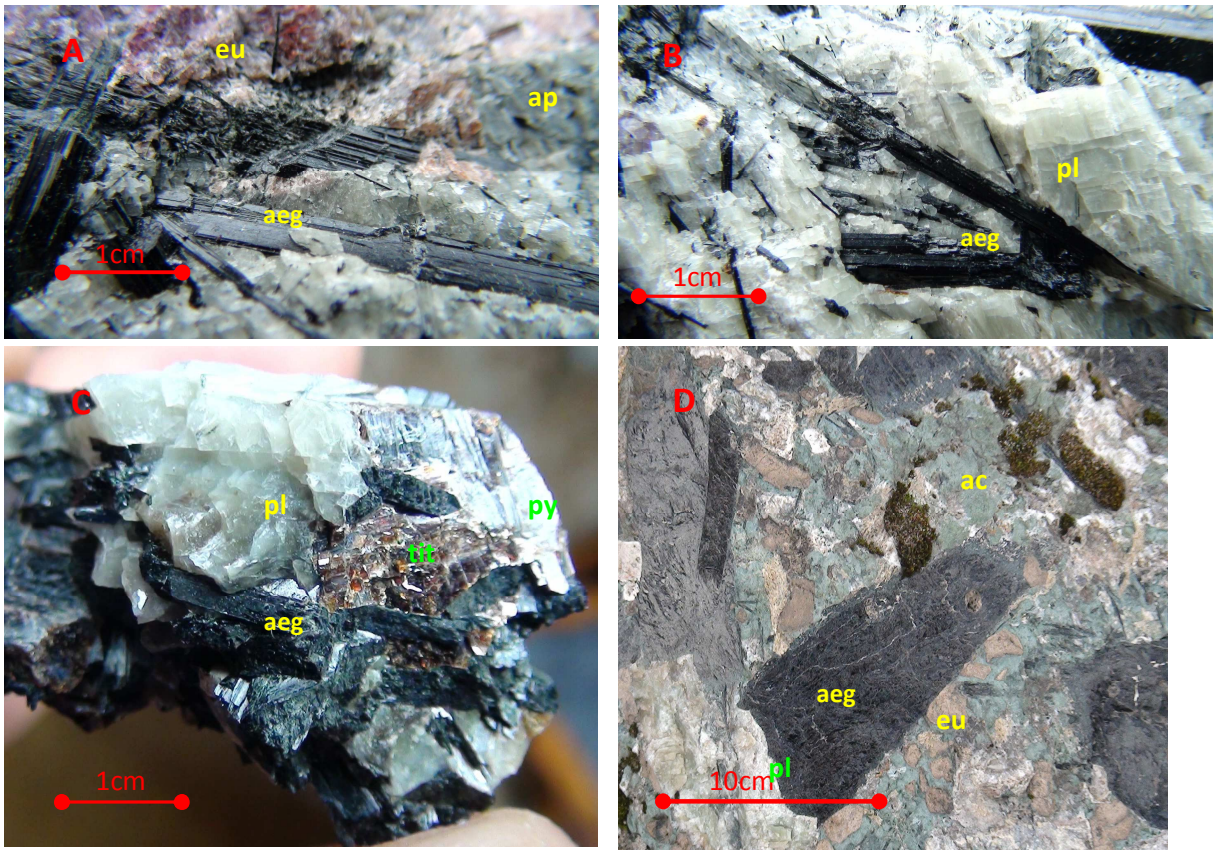

Fig. 1. Photographs of typical syenites from the study area: A. eudialytes and aegirine with apatite crystals, B. aegirine with plagioclases, C. titanite, sulphide on the plagioclases and aegirine, D. an example of pegmatite with giants crystals of aegirine, acmite, eudialyte and plagioclases (aeg-aegirine, ac-acmite, eu-eudialyte, pl-plagioclase, ap-apatite, tit-titanite, py-pyrite).

3. RESULTS

The samples from the Khibina Massif were collected in the center part of the mountains and Malaya Belaya Valley. In these areas are present syenites – „khibinites", massif aegirine syenites, urtites with nepheline-apatite ores, trachites and veins rocks [10] like microsyenites, tinguaites, melteigites and carbonatites.

Massive syenites are gray-pink colored rocks with visible a plagioclases and dark aegirine crystals with eudialyte, iron oxides, titanite and nepheline. They usually have holo-crystalline structure, compact, disordered texture (Fig. 1a-d). Plate minerals build rock background crystals of albite and orthoclase and some aegirine-acmite, nepheline and apatite crystals. Between these minerals are eudialyte (Fig. 3b) and ore minerals, such as ilmenite, titanite (up to 10% vol.) and riebeckite, arfvedsonite, pyrite, magnetite, astrophyllite (Fig. 3d), lorenzenite, usually not exceeding 5% by volume of rock. Astrophyllite usually crystallized in radiant forms, like aegirine called astrophyllite suns.

Massive aegirine syenites are gray-black rocks with visible aegirine crystals and plagio-clases accompanied orthoclase, iron and titanium minerals and apatite with nepheline accessory (Fig. 3a). They usually have holocrystalic coarsely crystalline structure, compact, incoherent tex-ture. These rocks in the microscopic image are composed of plagioclase and orthoclase crystals that form the backdrop of the rock. The close proximity of these minerals is aegirine, which usually forms aggregates of crystals fill the remaining spaces between the feldspars. Near these crystals are present apatite, nepheline, ilmenite, titanite, magnetite and titanomagnetite with sulfides (pyrite). It is present also arfvedsonite, aegirine-augite, and nepheline.

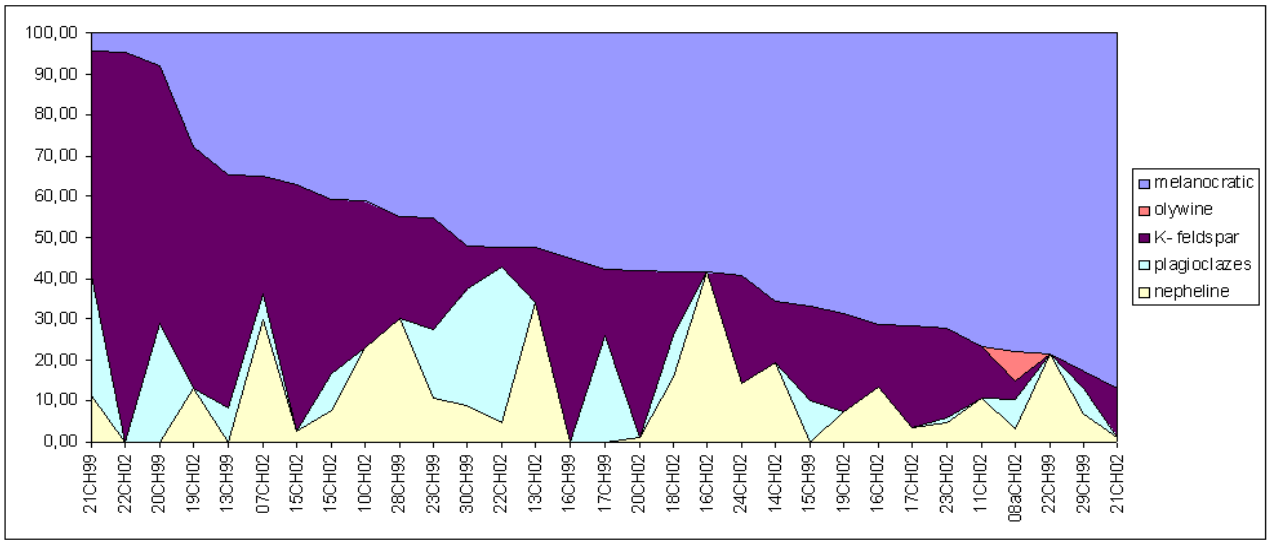

Fig. 2. Planimetric characteristics of the Khibina syenites.

Nepheline-aegirine urtites are gray-black and greenish rocks with visible in the background diced nepheline rocks and the accompanying plagioclases, aegirine, acmite, accessory and ore minerals (Fig. 3c). These rocks have less coarsely crystalline pseudo-trachyte structure. In the background are visible nepheline crystals which are sometimes up to 35% by volume of rock. Between nepheline are aggregates of various sizes aegirine crystals of up to 25% by volume of the rock. Other minerals are minerals ore, ilmenite and titanite mainly, acmite as well as apatite, plagioclase (albite). In microscopy nepheline usually has a first-order interference colors, being gray mineral, in the light of unpolarized usually colorless with a characteristic cube construction.

Apatite-nepheline ore rocks are brownish-green color (sometimes tinged with red). They are massive rock, usually constructed from ankle nepheline crystals (approx. 35%) between which developed titanite crystals (up to 35%) and apatite (up to 30 vol. %, Fig. 3e). Macroscopically nepheline crystals may have color gray or light pink. In the interstitial of these minerals meets ilmenite and arfvedsonite, aenigmatite, and aegirine, acmite.

Trachytes are gray color rocks with fenocrystals of K-feldspar (Fig. 3f). They have a porphyric structure, compact, incoherent texture. Orthoclase fenocrystals forms coming to 1 cm, usually twinning albite accordance. Next to feldspar appear individual crystals of augite with visible sieve structure filled by minerals background (e.g. orthoclase, coexisting with aegirine, acmite).

Analyzed rocks from Khibina are characterized by relatively large amounts of dark minerals (mainly pyroxenes and ore) and a relatively large amount of K-feldspar (orthoclase) accompanied by plagioclase (mainly albite) and nepheline. In the mineral veins (melteigites) share of dark minerals is the biggest and also exist olivine (Fig. 2).

3.1. Detailed analysis of the phase minerals from the studied rocks

By analyzing rock samples from Khibina massif focuses on the observation of the rock-forming minerals such as feldspar and feldspathoids which pyroxene and amphibole and accessory minerals such as apatite, nepheline, eudialyte, arfvedsonite, riebeckite, aenigmatite, ore minerals phases detailing such as impurities and inclusions containing rare earths

3.1.1. Feldspars and feldspathoid

To the rocks of Khibina Massif feldspars belong mainly series of acidic plagioclase albite, oligoclase and K-feldspars such as orthoclase (Fig. 10). In some feldspars with admixture may appear inclusions Sr. 442 analysis of the Khibina Massif feldspar indicates that most of them are Na-plagioclase. Albite with orthoclase is over eighty

percent frequency of appearing in the analyzed rocks. Other plagioclases as andesine or oligoclase have frequency of approx. 15%. Ca-plagioclase group prevalence of approx. 4%, and most often associated with the mineral veins.

Together with feldspar in Khibina rocks recorded in the presence of feldspathoides such as nepheline, it constitutes 43% of the surveyed all aluminosilicates Na and K (343 analysis, fig 12). In the khibinas nephelines can be present as small inclusions containing Ti and Fe, Sr and Nb. Beside feldspar crystals apatite appears. It is generally calcium apatite (Fig. 11), often with an admixture of fluorine and chlorine. 78 analyzed apatites they also noticed numerous inclusions of strontium and rare earth elements. In some samples, together with apatite are found fluorite (31 analyses of these minerals, Fig. 21).

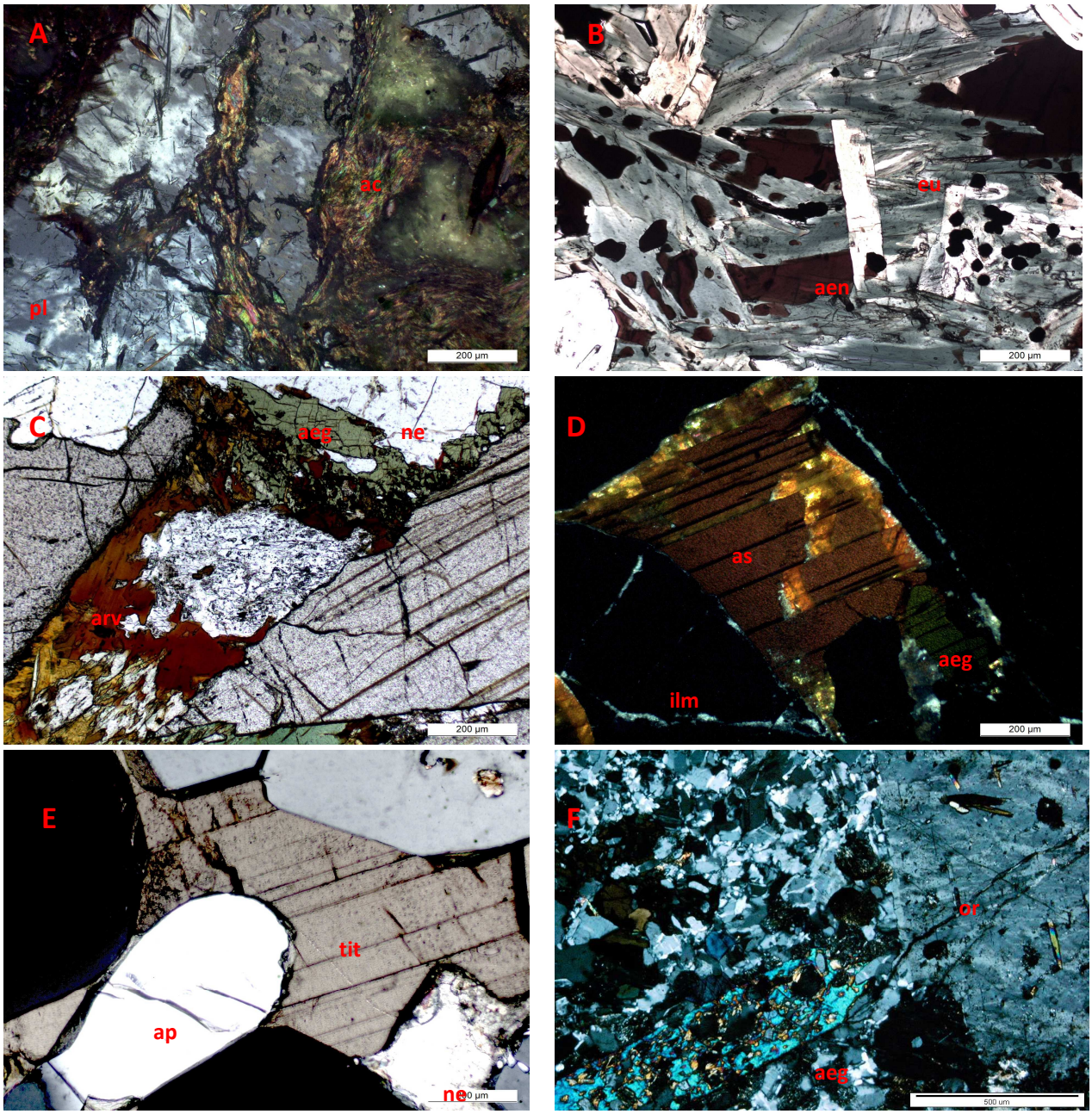

Fig. 3. Example of syenites microphotographs from the Khibina: A. acmite on the plagioclase crystalls, B. aenigmatite crystals on the eudialyte, C. tytanite with aegiryne and arfvedsonite witch nepheline (urtite), D. astrophyllite crystals with aegirine near ilmenites, Ore rocks: E. titanite with nepheline and apatite. F. trachytes: orthoclase fenocrystals with aegirine (Cross light: C-E, reflected A-B, 1N: C, E, Nx: A, B, D, F; ac-acmite, pl-plagioclase, eu-eudialyte, aen-aenigmatite, aeg-aegirine, arv-arfvedsonite, as-astrophyllite, ilm-ilmenite, or-ortoclase).

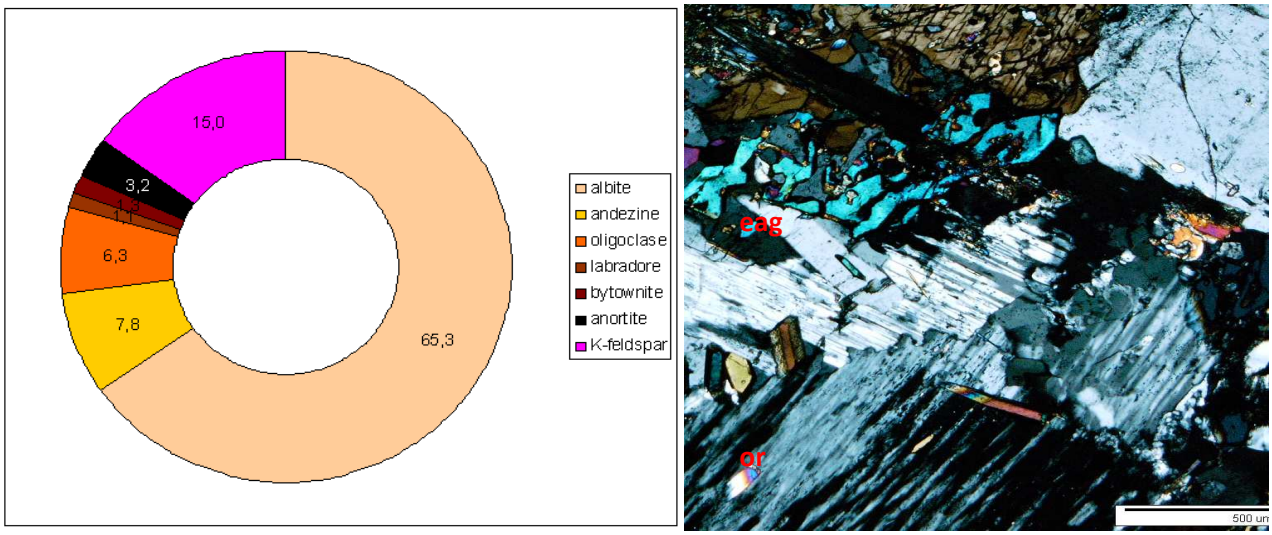

Fig. 4. The frequency of occurrence of different types of feldspars (in left) in the studied minerals from the Khibina syenites and microphotography sample obtained in the polarizing microscope in transmitted light Carlsbad twinning orthoclase in syenite (in right).

In addition to these minerals are abundant eudialytes (Fig. 13) and lorenzenites (Fig. 14). There are accessory minerals nature, but in the case of Khibina often present in significant amounts in rocks acting part approx. 10 vol.% or more. Eudialytes of excommunication is colored red or cherry. Intensive red crystals it named by "Loparian blood". In the described rocks eudialytes have polymorphic and a single, xenomorphic crystals, size of up to several cm. Characteristic for this mineral d_{hkl} are: 2,853 (100); 2.979 (97); 4,339 (71); 3.189Å (49). Some of the mineral crystals exhibit zonal construction. Eudialytes were analyzed in 166 cases. In addition to the minerals of iron, sodium, calcium unusual additives are sometimes Nb. The lorenzenites (total of 51 analyzes made) in addition to sodium and titanium have a Sr and Nb.

3.1.2. Pyroxenes and amphiboles

Pyroxene from the studied rocks is very common. They are found in all types of rock samples from Khibina. In the samples of syenites, there are numerous crystals of aegirine, augite and sometimes with an admixture of sodium or titanium (aegirine-augite). Very common processes involve the aegirinization of augites. Ti and Na grow share in the border of crystals at the expense of magnesium and aluminum. Fenitization of the augites is associated with the processes of crystallization rocks in alkaline surfaces are especially clear in the rock veins, where there is a corrosion of pyroxenes and the resulting second generation rich in iron and sodium. Number of analyzes of samples with pyroxenes is 693, of which aegirine and augite steppe 389 times, causing changes in the distribution of titanium and sodium within these phases. Pyroxene rocks constructed mainly as automorphic or sub-automorphic crystals formed from large specimens to 30 cm length and form felted, needle like acmite sometimes also crystallizing in a radial form. In the vicinity of these minerals are often seen symplectite adhesion of pyroxene with ilmenites occurring, arising from the demixing of solution. Pyroxenes companion to a much smaller share of amphibole crystals.

Among the pyroxenes most common pyroxene is aegirine and aegirine-augite. A number of orthopyroxenes is represented by enstatite, which will in principle present in melteigites. In other rocks are found veins mainly diopside and ferrosalite addition (Fig. 5, 15-18). Augite and aegirine, were studied in detail. These minerals are developed in the form of columnar crystals of up to 0.5 m on average, not reaching the size of 5 mm.

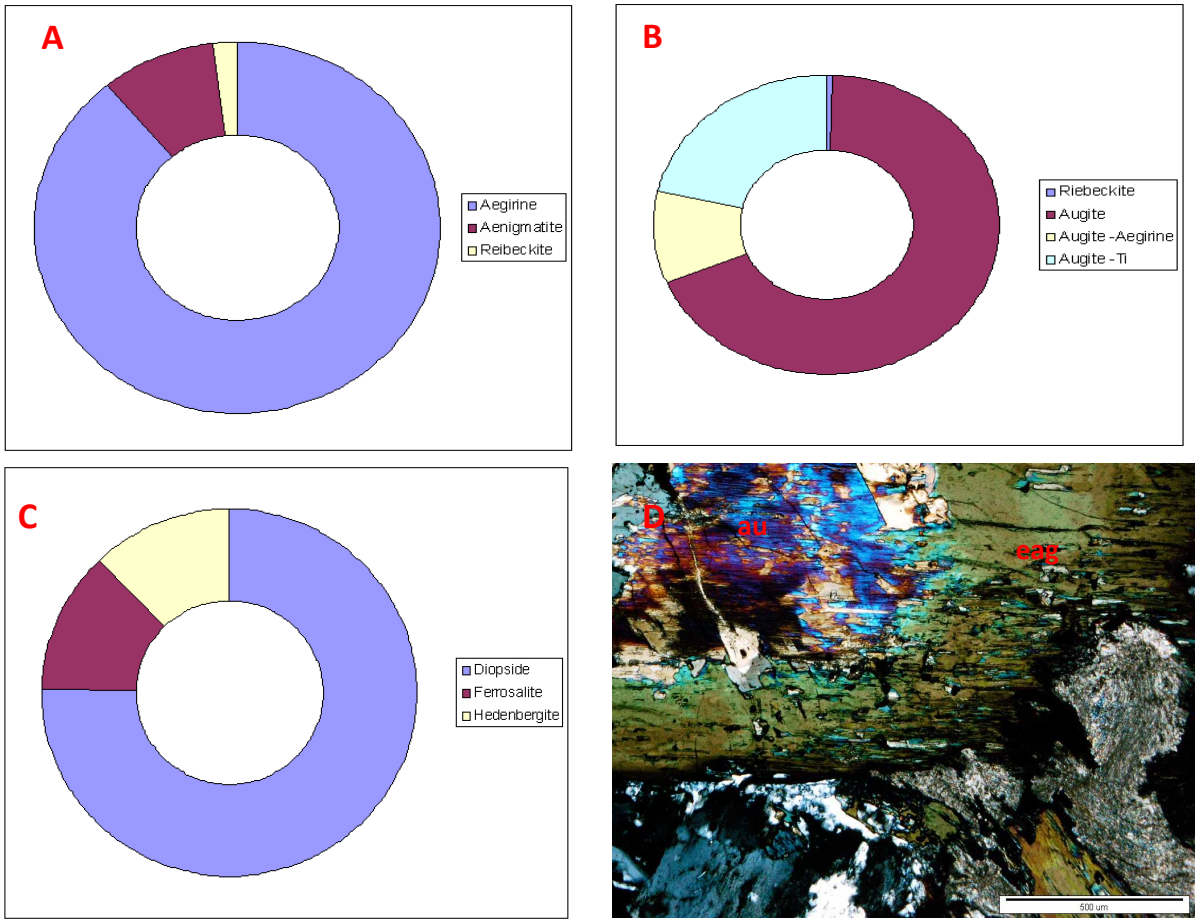

Fig. 5. The diagrams of pyroxenes (A: Na-Ti pyroxenes, B: augites and other rich in Al, C: Mg-Fe pyroxenes) and sample microphotograph (D) showing aegirinizing augite crystal (crossed pollars, cross light).

The figure below shows the diffraction pattern of the aegirine-augite (Fig. 6a), and its infrared spectrogram (6b). Usually, they are represented by a aegirine molecule, sometimes aegirine-augite, there are also the transition from augite to aegirine continuously (in the form of crystals fouling, Fig. 2f). The figure below shows the diffraction pattern of the aegirine-augite (2a), and its infrared spectrogram (2b).

Silicates chains relatively common in the rocks occurs astrophyllite (Fig. 1d), which macroscopically characterized by the color yellow or light brown. Creates a radiant focus (i.e. astrophyllite sun), rarely sheaves. In a study in the micro inclusions were found in pyroixenes solid containing small quantities of Ta and Nb. Of the amphibole is present small single crystals of riebeckite and arfvedsonite accompanying minerals such as ilmenite, titanite. Mica minerals are accompanied by biotite and found mainly flogopite in the veins rock to form a crown there reaction (Fig. 6e, 19). Next to mikas in the veins rocks the also present olivine, mainly magnesium (Fig. 6f, 21), as enstatite undergoing corrosion. In the immediate vicinity pyroxene minerals are common ore and numerous inclusions, which are described below.

3.1.3. Ore minerals

Among the ore minerals analysed in the Khibina massif found the vast majority of the occurrence of ilmenite and titanite and magnetite (Fig. 7, 22-25). At the 606 surveyed ore minerals ilmenite is the number of 57,92%, titanite 16,34%, magnetite 10.73%. Ilmenite occurs in rocks quite commonly as sometimes significant share in the ore minerals of rock. Ilmenite crystals sometimes reach 2-3 cm in size. The veins rocks sometimes it has a characteristic structure of disintegrating, will phase crystallizes as a result of the spinel disintegration. In ilmenites are present numerous

of ad-mixture elements such as V, Nb, Ta (Fig. 25). Together with ilmenite co-occurs titanite, which, along with ilmenite occurs most frequently in these rocks. Titanite crystals are sometimes ad-mixture Nb, Ta, W (Fig. 22).

Titanite from Chibina most often occurs in the form of an envelope resembling a flattened poles having the form of a wedge-shaped cross-section (Fig. 8). Although it is often in the form of both hypomorphs (Fig. 8b). Interesting is also lamellar twinning titanite visible in transmitted light. Average crystal size is up to 5 mm, although there are signs exceeding the size of 5 cm. In the vicinity of titanite noticed ilmenites, magnetites and enriched with pyroxene and amphibole titanium.

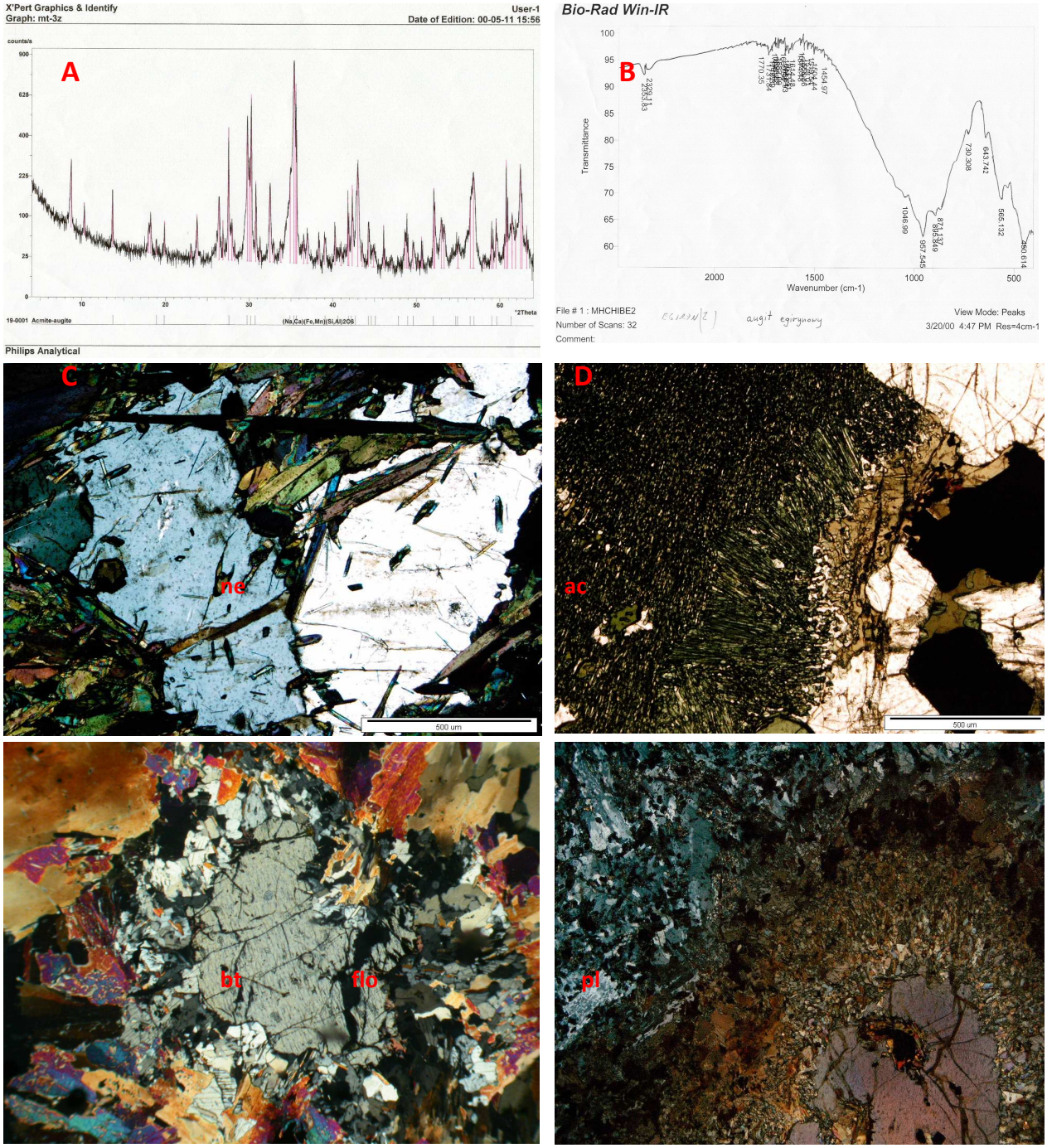

Fig. 6. Aegirines: A - XRD powder pattern, B - IR spectrogram C - microphotographs of aegirine against the nepheline, D - example of acmite, E, F-crown reaction at about orthopyroxene and olivine mainly composed of micas (C-E cross light F-reflected light, crossed pollars).

Often in the company of titanite are ilmenites, but there are also titanomagnetite (although the latter are secondary to changes in maghemite and hematite). In addition titanite and minerals found in rocks magnetite (Fig. 24) and rutile (3.63%; Fig. 23).

In addition to these minerals are relatively often encountered various kinds of sulphides such as pyrite 3.96%, chalcopyrite, pentlandite and arsenopyrite, occur these rocks in an amount of less than 1% (Fig. 26). In some samples also noticed chalcozine and sphalerite minerals. Among these phases ore minerals are also found Sr (3.30%), Nb, Pm (about 2% and admixtures of magnetite V_2O_5 in number 5%, and thallium 2-3%). These are usually solid inclusions containing these elements. It was also found incidence löllingite (Fig. 27).

Along with numerous rock-forming and accessory minerals minerals in rocks are found zirconium silicates, usually represented by a Zr but microprobe analysis showed that some of these phases has a small admixture of Na (Vlasovite?, Fig. 29). Zircon like many other ore minerals mostly revolve around piroxenes or other dark minerals.

Between the femic such as augite, aegirine and some ore minerals in syenites occurs in many different kinds of inclusions. Part of these phases are accompanied microveins, cracking the rock, which fills the rock material similar content sometimes enriched in carbonates and sulphides (eg. the aforementioned sphalerite).

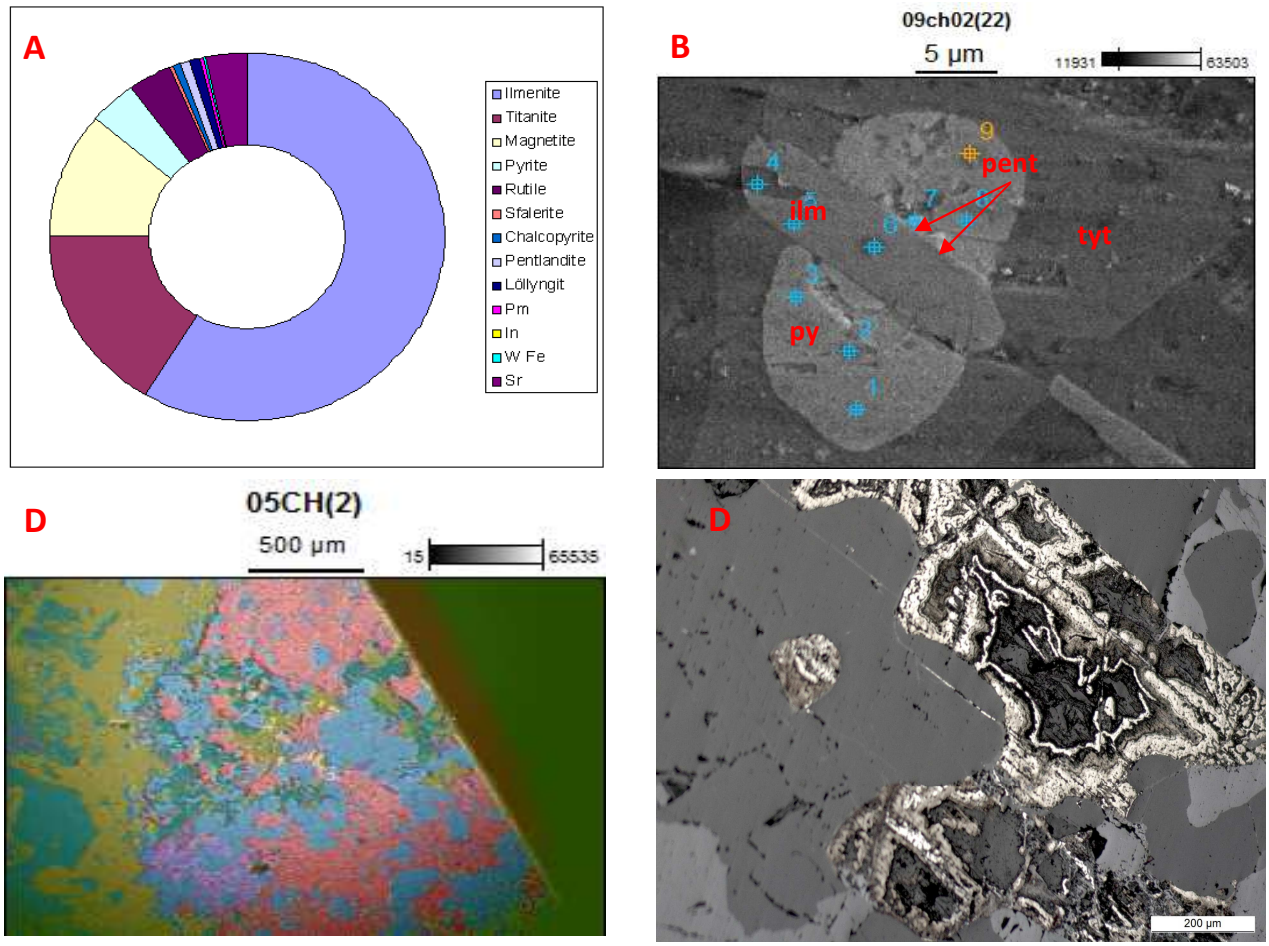

Fig. 7. Documentation showing the presence of ore minerals in the analyzed rocks: A - block diagram indicating the percentage of ore minerals. B - back scattering microphotograph (BSE) from electron microscopy of ilmenite crystal, pyrite and pentlandite overgrown against titanite, C - phase analysis performed using scanning electron microscopy coexistence of sulphides and oxides (blue - ilmenite, violet - chalkopyrite, pistachio - aegirine, green - augite), D - microphotograph of chalcopyrite in syenite (reflected light).

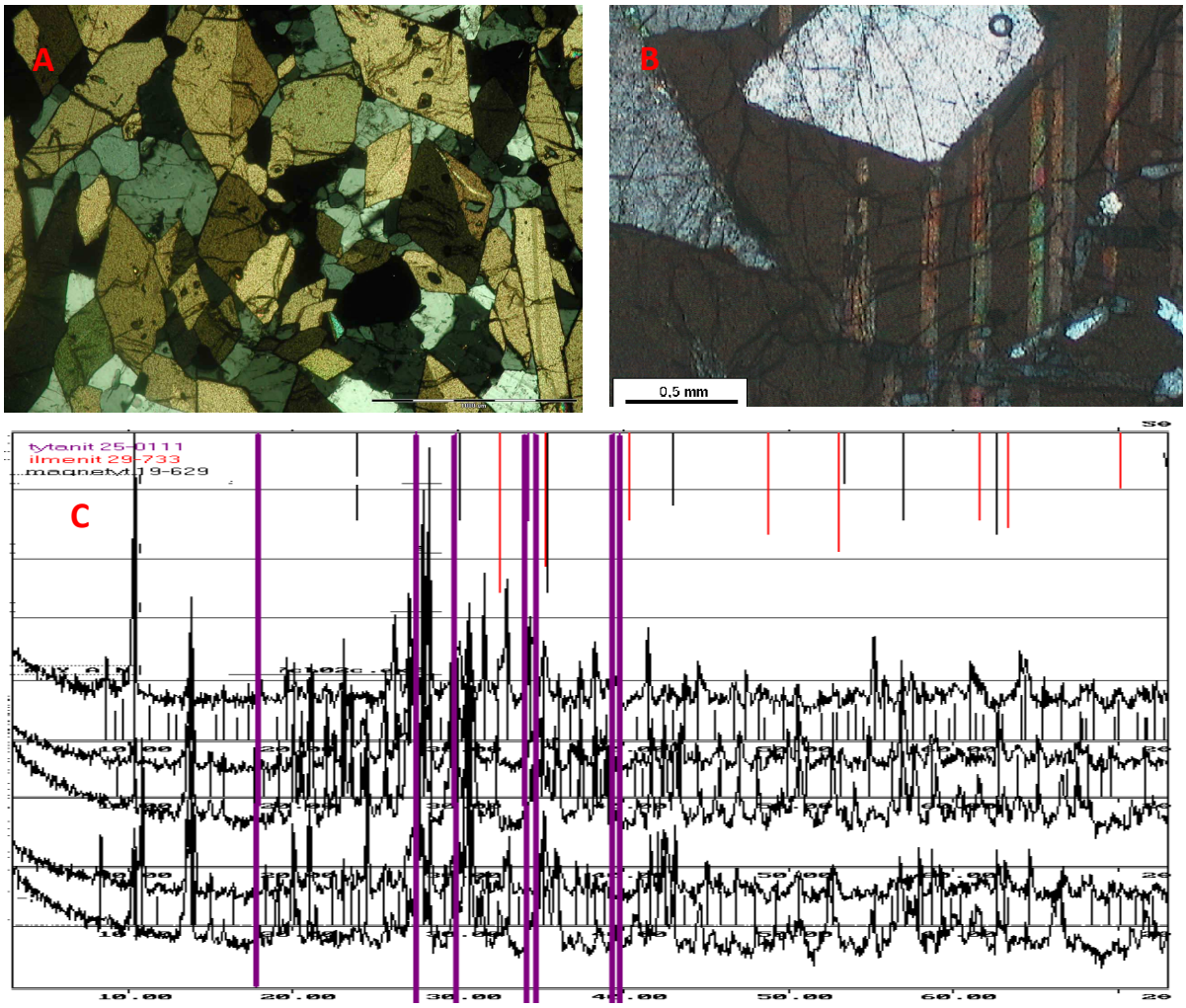

Fig. 8. Titanite cystalls in ore rocks - A, sectors construction in titanite from nepheline syenites - B (A, B microphotograph of the tytanite in cross light, crossed pollars), C - XRD powder patterns of titanites with ilmenites from the studied rocks.

Table 1. Results of the analyses in the microphase illustrated in Fig. 9.

phase	sample	C	O	Na	Mg	Al	Si	S	K	Ca	Ti	Mn	Fe	Zn	Sr	Ba	La	Ce	As	Nb	Ag	Cd	Nd	U
barite	06CH(4)_pt1	3,24	24,17					13,52		0,18						58,88								
inclusion	06CH(4)_pt2	5,05	34,55							3,63					13,66		25,36	15,73						
calcite	06CH(4)_pt6	5,08	45,36							47,19		0,5												
augite	06CH(4)_pt4	1,8	33,57			3,36	17,45		7,33		0,59	1,3	33,7	0,44										
aegirine	06CH(4)_pt3	1,39	36,64	7,83	1,45	0,37	25,24			3,98	1,5	0,42	20,07											
aegirine	06CH(4)_pt5	1,49	36,4	9,13	0,74	0,29	25,15			1,64	0,52	0,27	22,72											
inclusion	06CH(22)_pt1	4,45	37,69								6,65	0,17	3,88	0,67					0,46	16,73		3,69		8,70
inclusion	06CH(22)_pt2	4,44	35,29								7,18		4,44	0,65						17,94			0,60	11,53
inclusion	06CH(22)_pt3	4,53	43,30								6,81		4,33	0,57						16,43		6,71		
inclusion	06CH(22)_pt4	2,67	38,33								5,16	0,71	11,47	0,47							2,80	7,48		
aegirine	06CH(22)_pt5	2,97	41,52	8,55	1,07	0,50	23,62			1,75	1,49	0,42	17,90											

In places such opportunities also meets sulfates such as barite and their company are numerous inclusions often contain relatively high concentrations of lanthanide elements (Fig. 9). Lanthanide elements (Lc, Pr, Ce) pass also others such as Sr. Inclusions containing uranium are also Nb, Cd and Ti-Fe, Zn admixture (Fig. 28). Probably uranium compounds present in these rocks in the form of oxides (probably uraninite). Such inclusions occur in these rocks often but not always have to have a composition as described above. However, given the nature of the Khibina intrusion rocks it is an area perspective to the presence of small aggregates of many different elements.

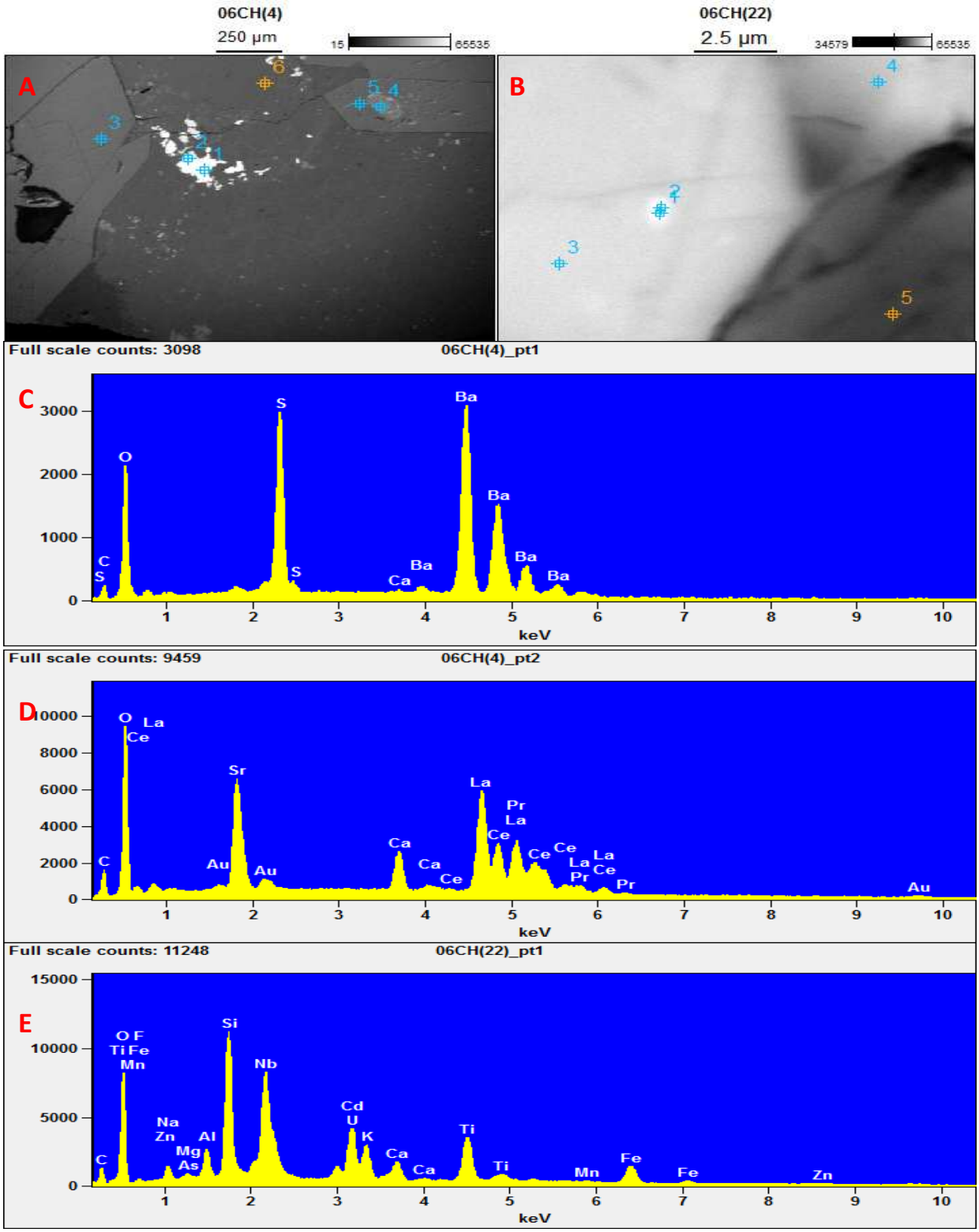

Fig. 9. Microphotograph (BSE) of the Barite (A) and Uranium (B) inclusions and corresponding EDS spectrum (C-E).

5. DISCUSSION

Khibina and Lowoziero Massifs are one of the types of rocks intrusions on the Kola Peninsula and in the N part of the Baltic Shield [1, 8]. It contained all kinds of syenite rocks a very unusual, unique instance of such a large area, which is on the surface of the ground, and can be, in principle, without limitation explore. These syenites are constructed in different petrographic varieties, according to the orientation of the intrusion. Mineralization that occurs in these rocks is diverse

and interesting. This applies both to the characteristics of the rock-forming minerals which accessory. Of particular interest are different kinds of impurities and inclusions comprising a numerous of elements including REE and radioactive. These additives are documented many of rock-forming and accessory minerals in the Khibina rocks. Particularly frequently repeated here are Sr admixtures (Fig. 30), which are present in many different minerals from Khibina. The different kinds of rocks inclusions are probably associated hydrothermal processes (barite-sphalerite-carbonatite association), and may represent the last stage of the formation of these mountains, while in the central part on the rocks formed in carbonate rocks [2].

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

Annex of the graphical presentation minerals using SEM-EDS method.

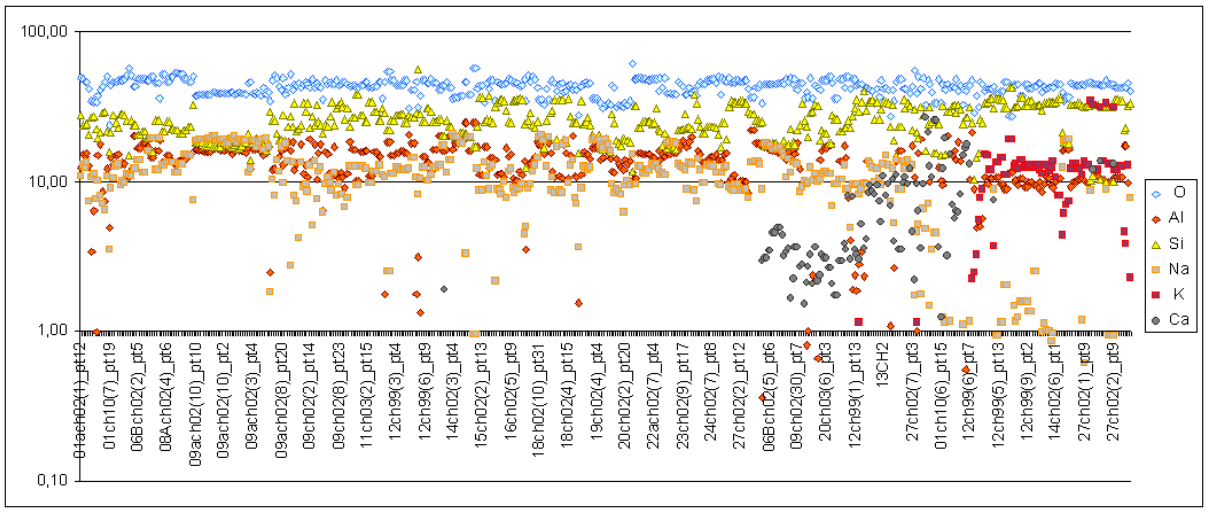

Fig. 10. The results of plagioclases microanalysis.

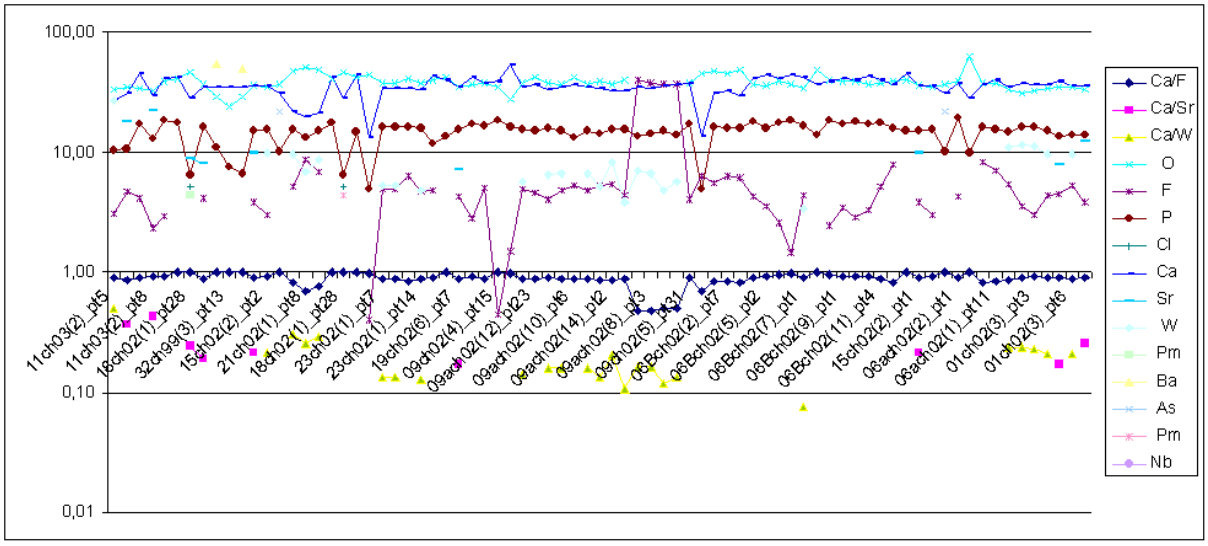

Fig. 11. The results of apatite microanalysis.

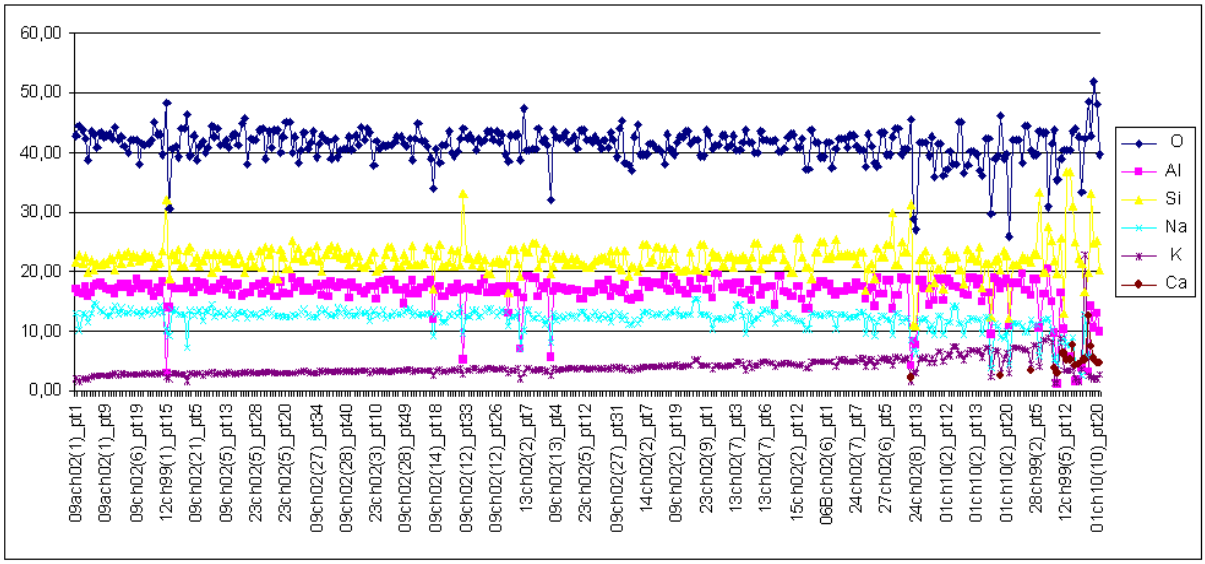

Fig. 12. The results of nepheline microanalysis.

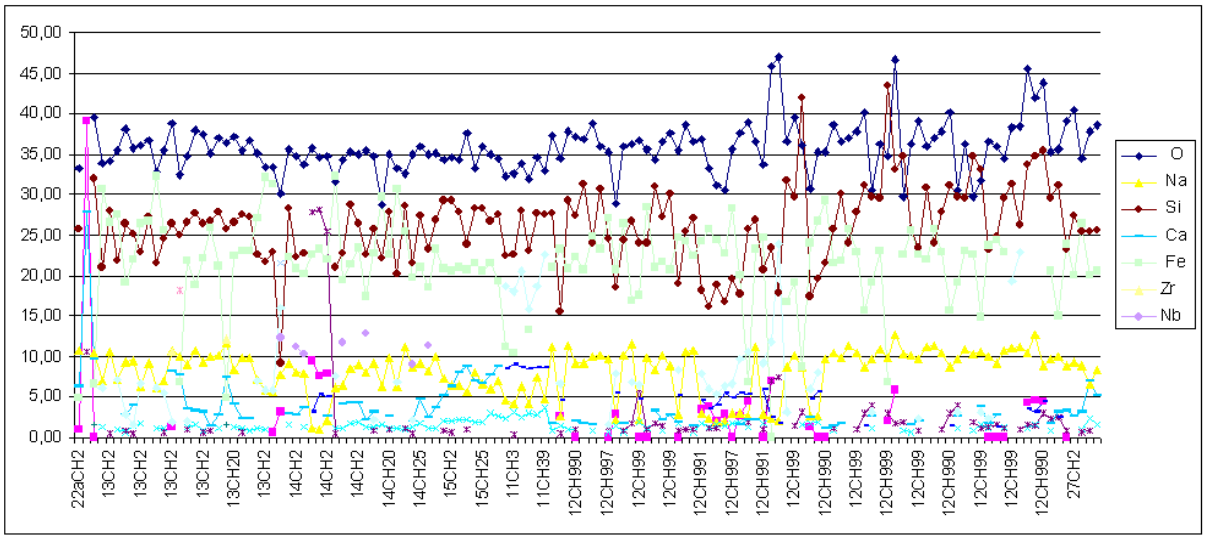

Fig. 13. The results of eudialyte microanalysis.

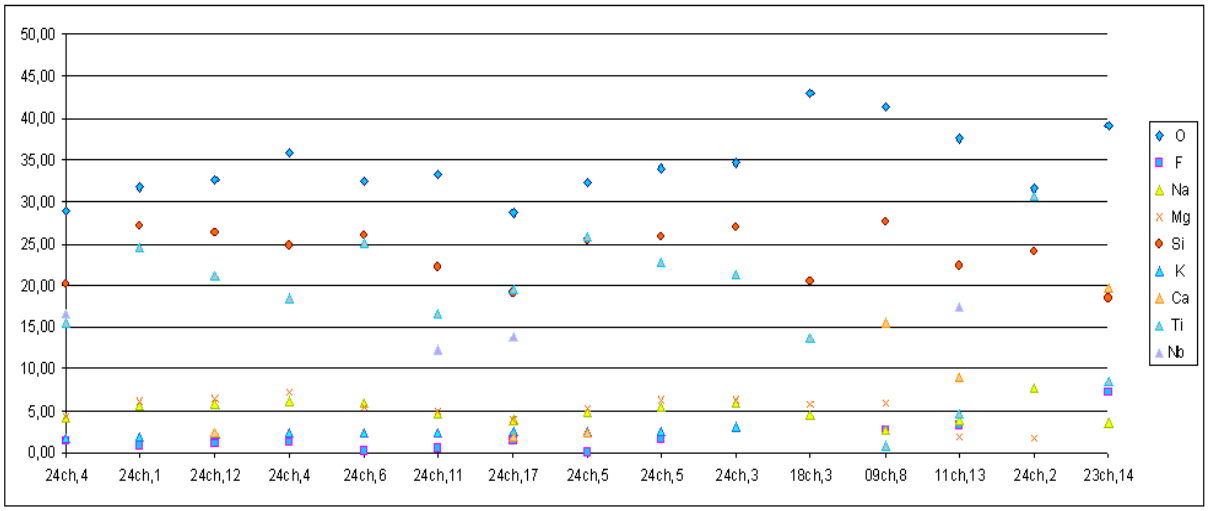

Fig. 14. The results of lorenzenite microanalysis.

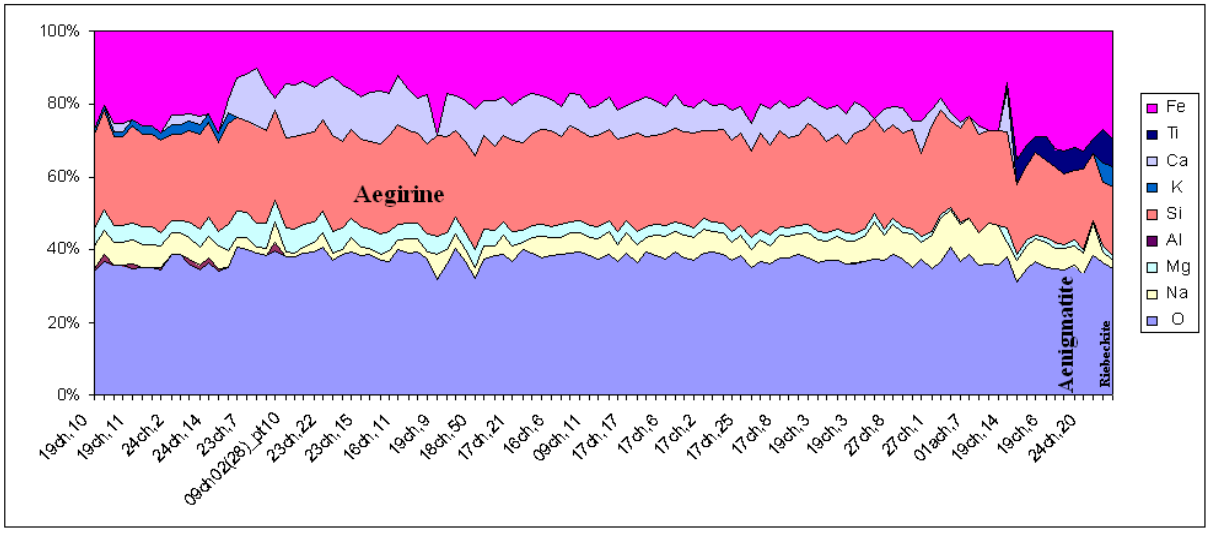

Fig. 15. The results of aegirine/riebeckite microanalysis.

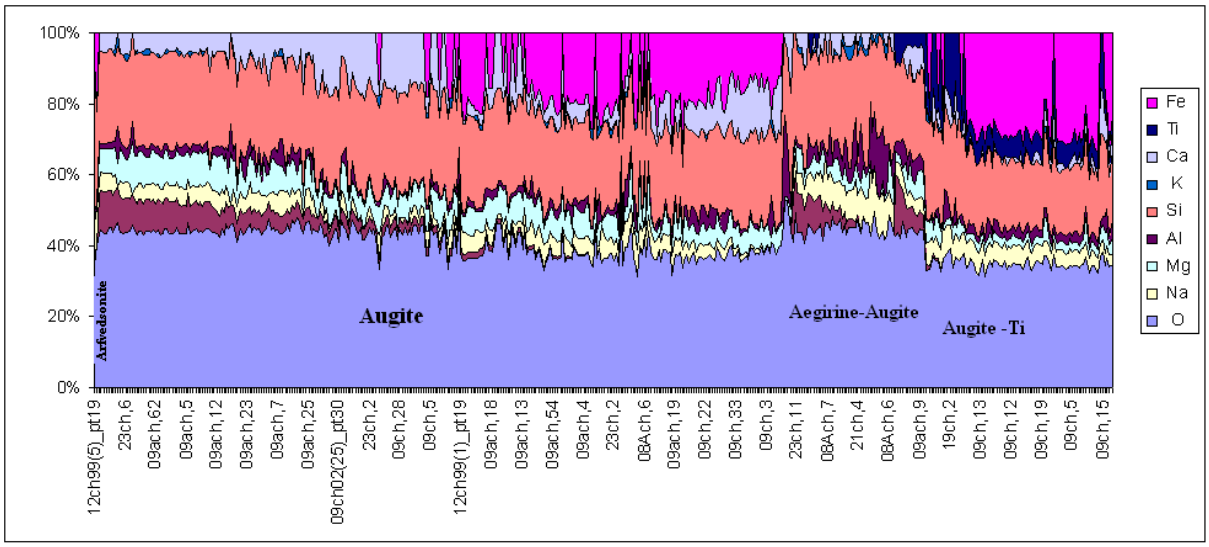

Fig. 16. The results of augite microanalysis.

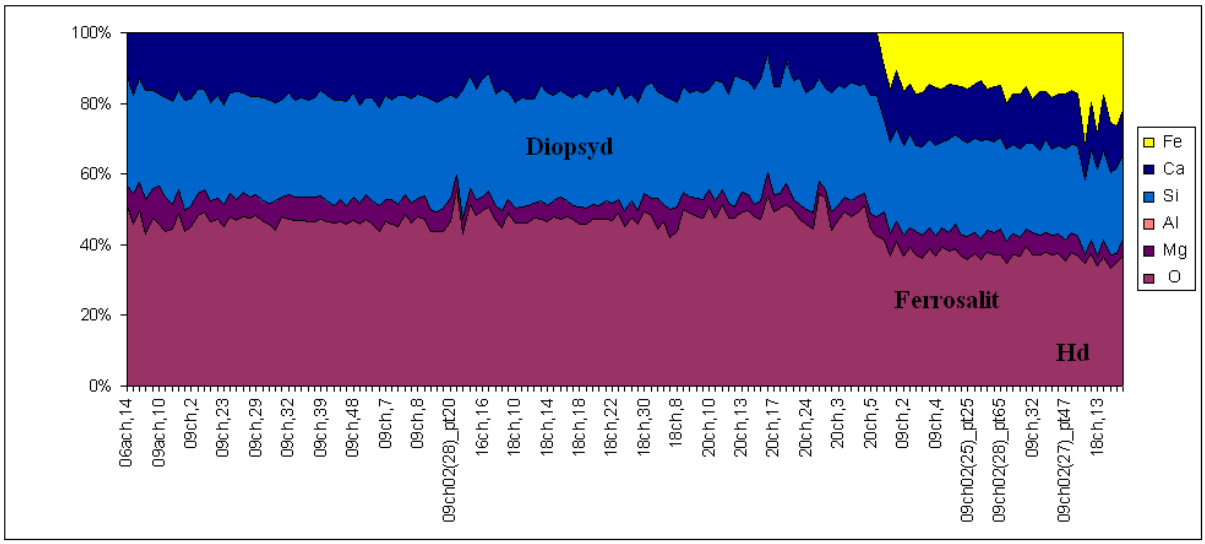

Fig. 17. The results of diopside-hedenbergite microanalysis.

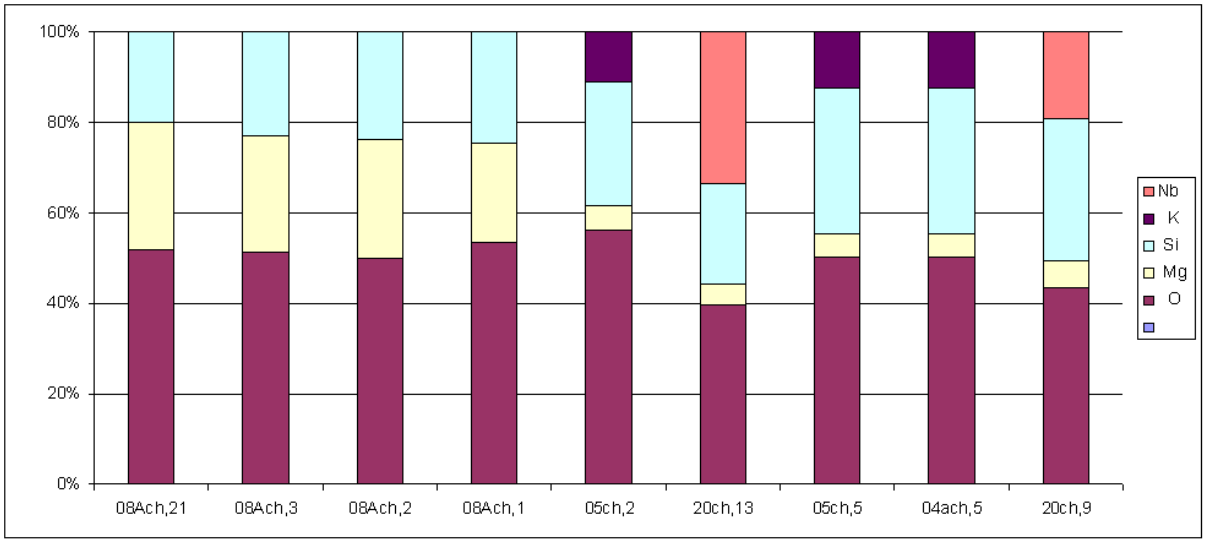

Fig. 18. The results of enstatite-hyperstene microanalysis.

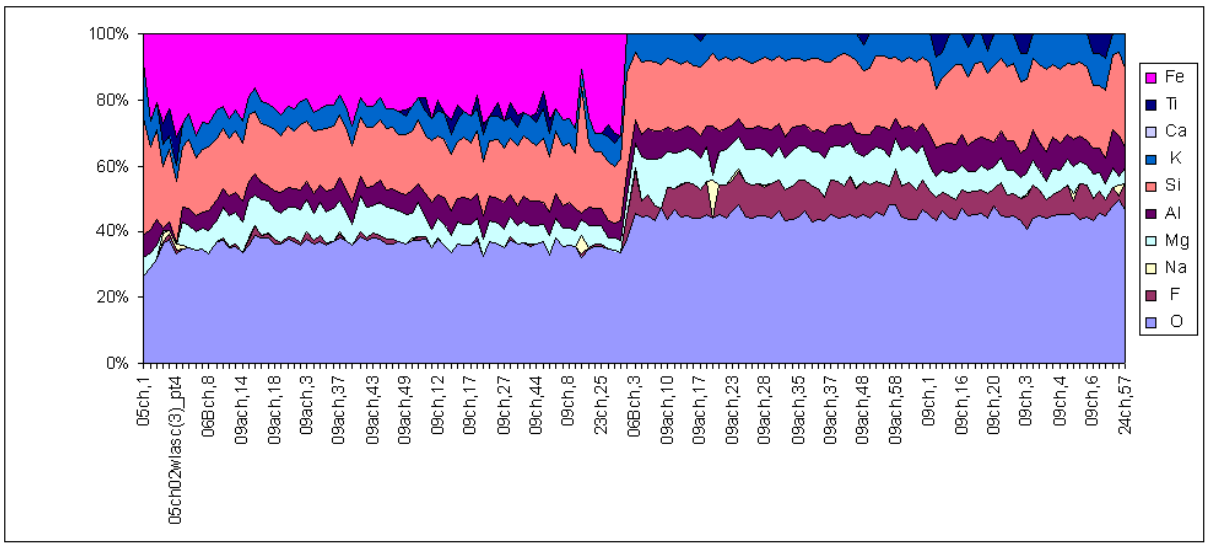

Fig. 19. The results of micas microanalysis.

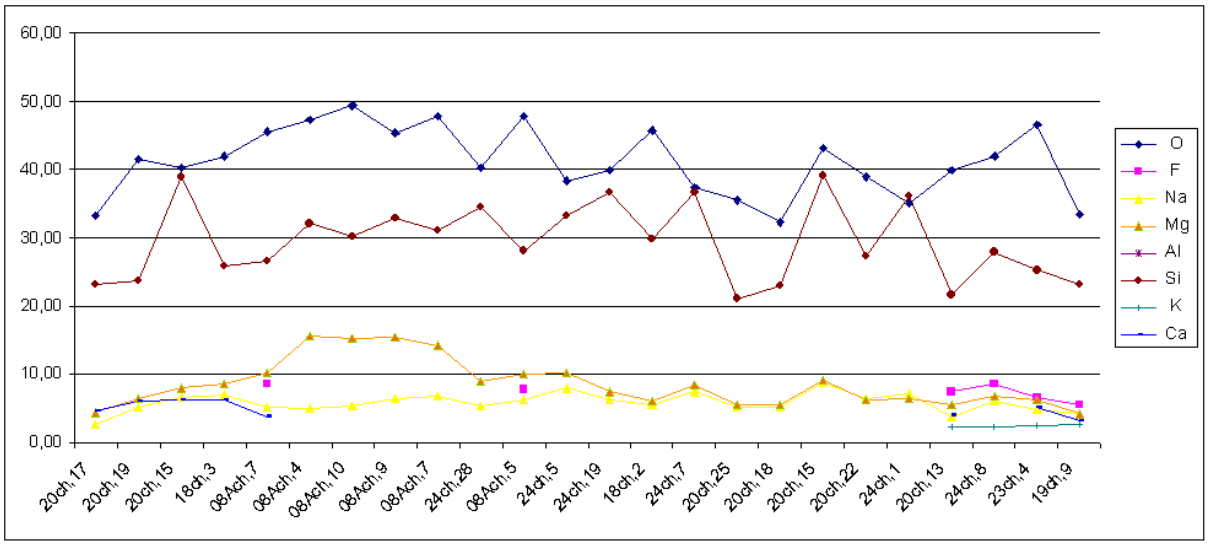

Fig. 20. The results of olivines microanalysis.

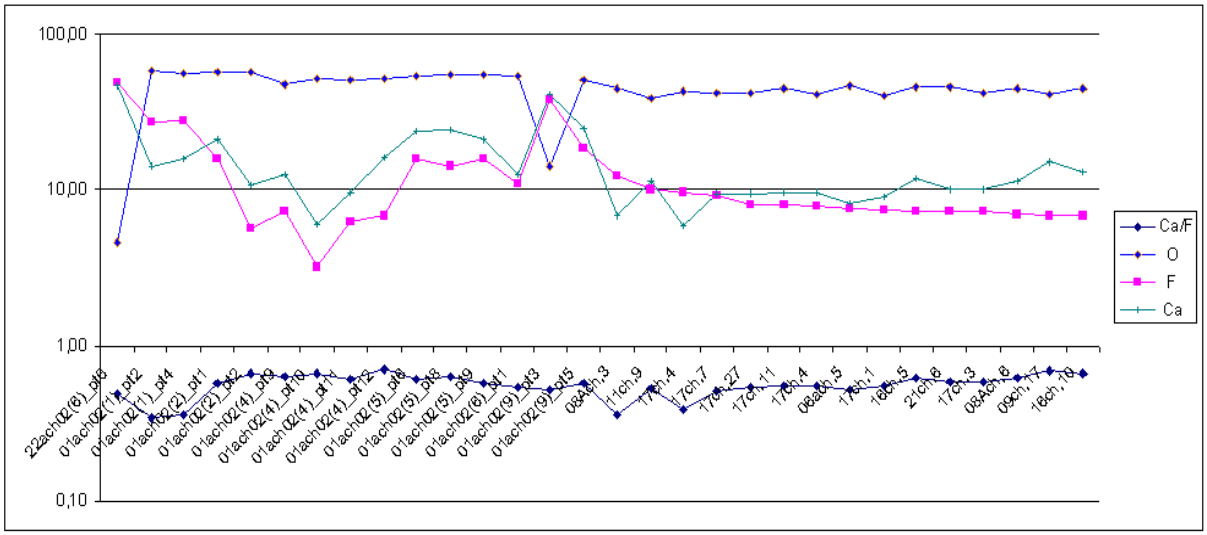

Fig. 21. The results of fluorites microanalysis.

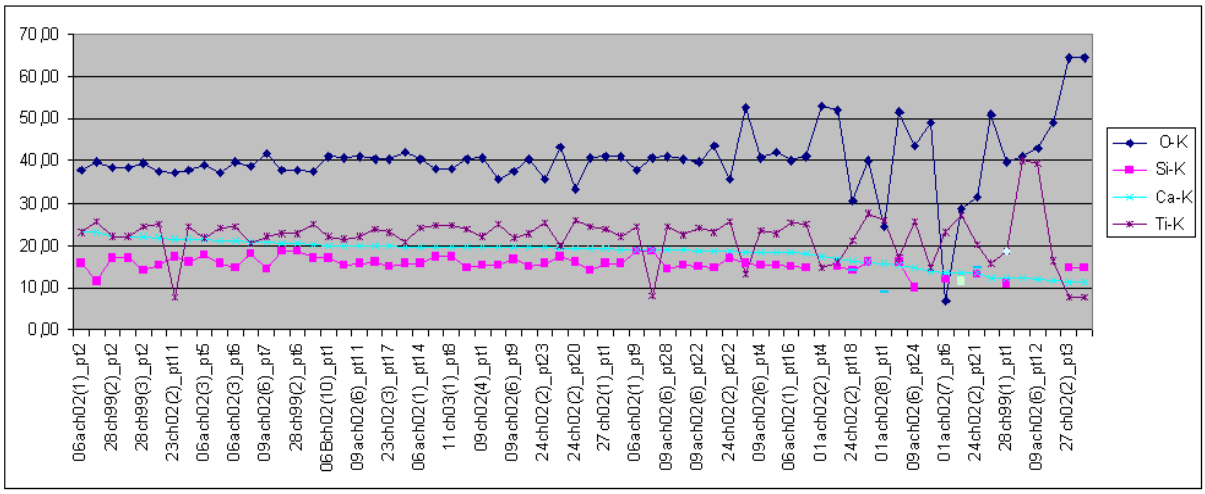

Fig. 22. The results of titanite microanalysis.

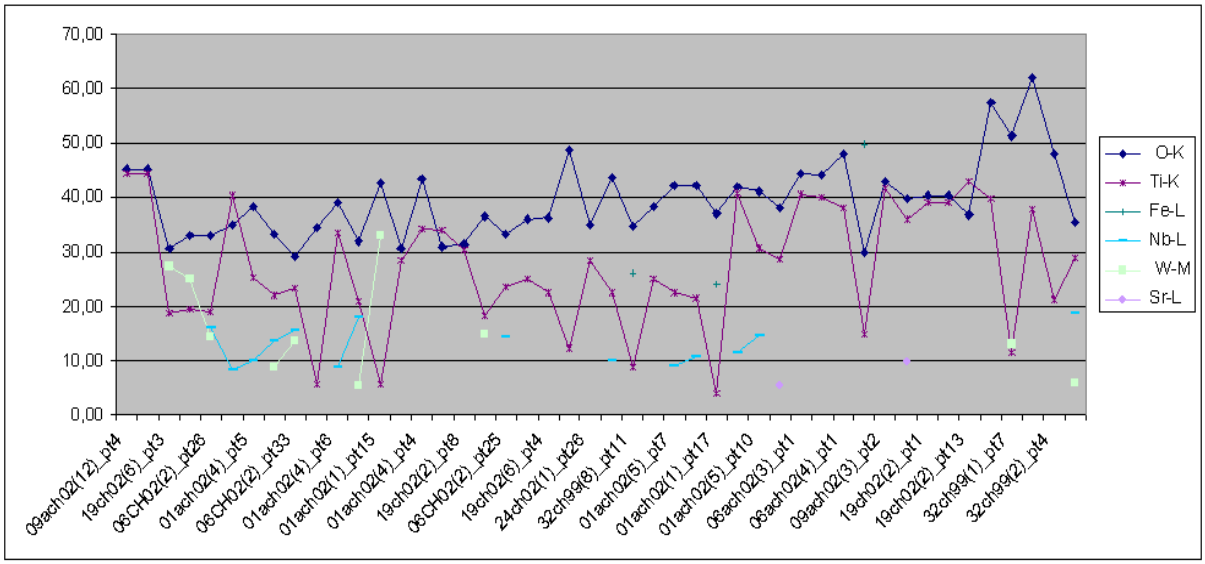

Fig. 23. The results of rutile microanalysis.

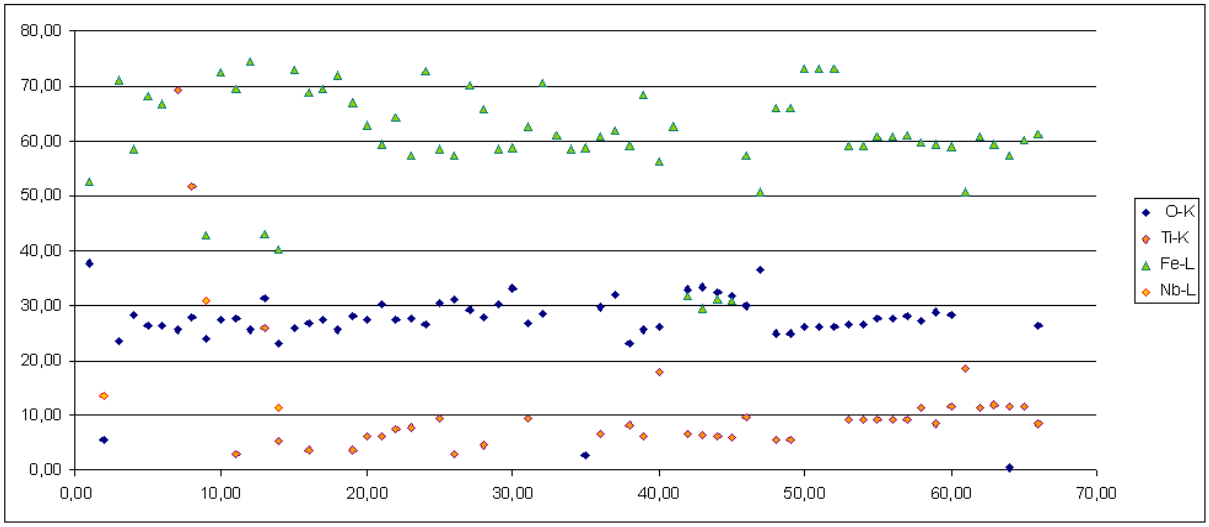

Fig. 24. The results of magnetite microanalysis.

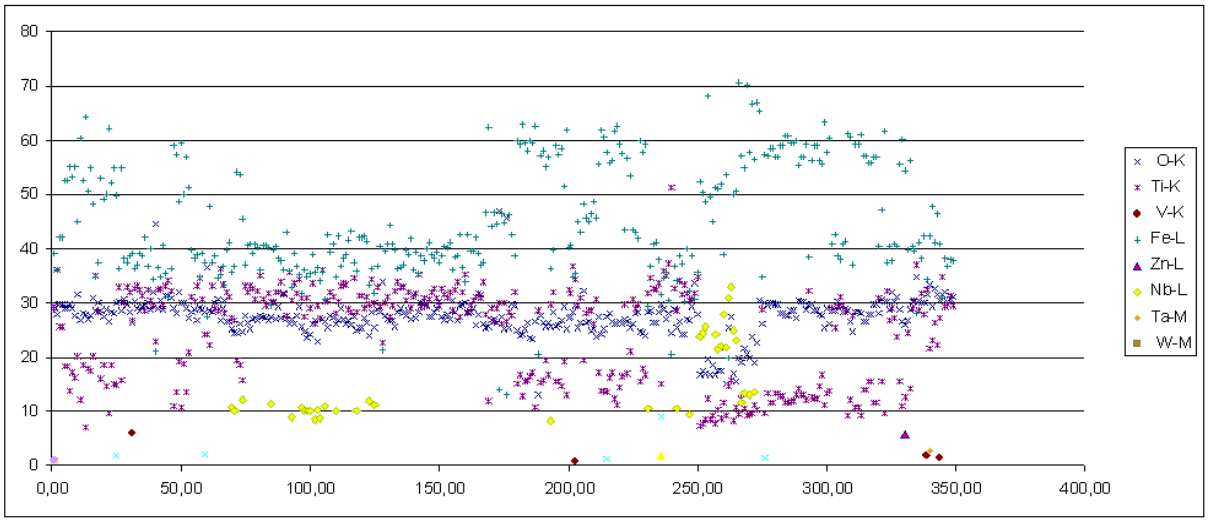

Fig. 25. The results of microanalysis of the ilmenite.

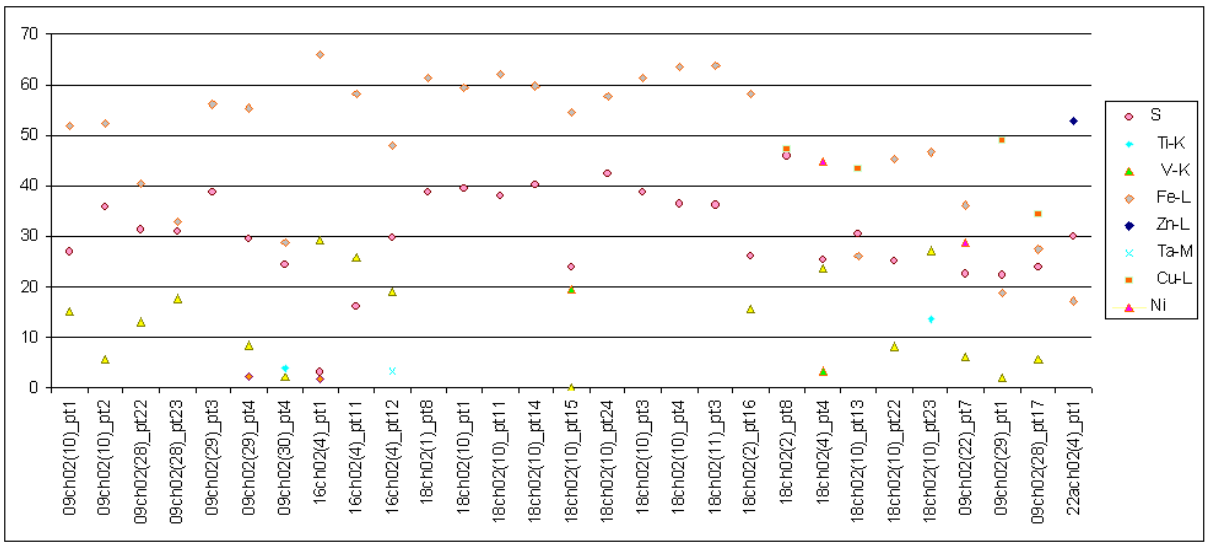

Fig. 26. The results of microanalysis of the sulphides: pyrite, chalcopyrite, pentlandite, chalcozine (18ch02(2)_pt8) and sphalerite (22ach02(4)_pt1-sphalerite).

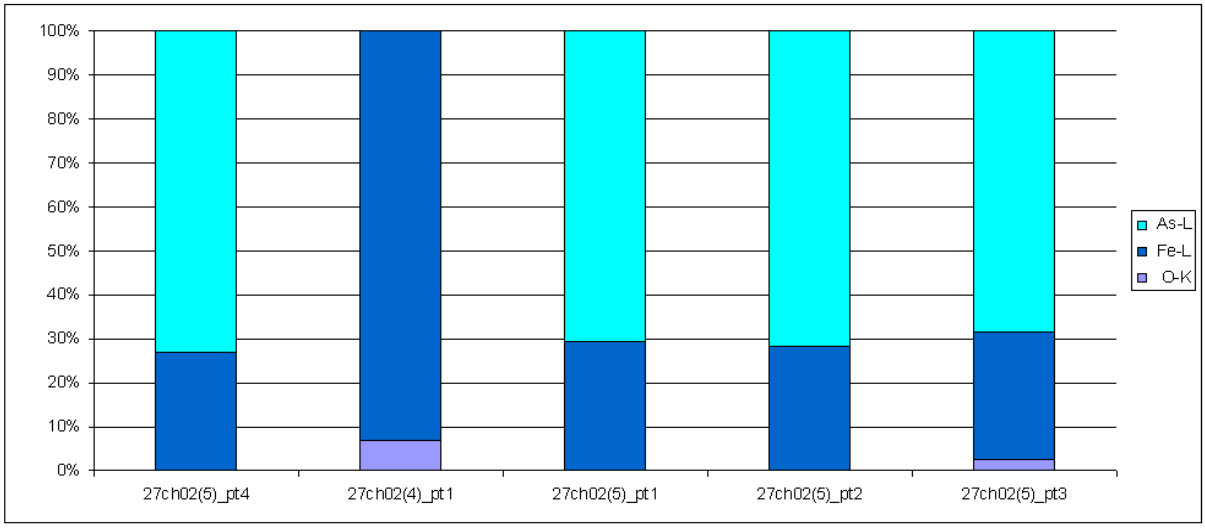

Fig. 27. The results of microanalysis of the löllingite.

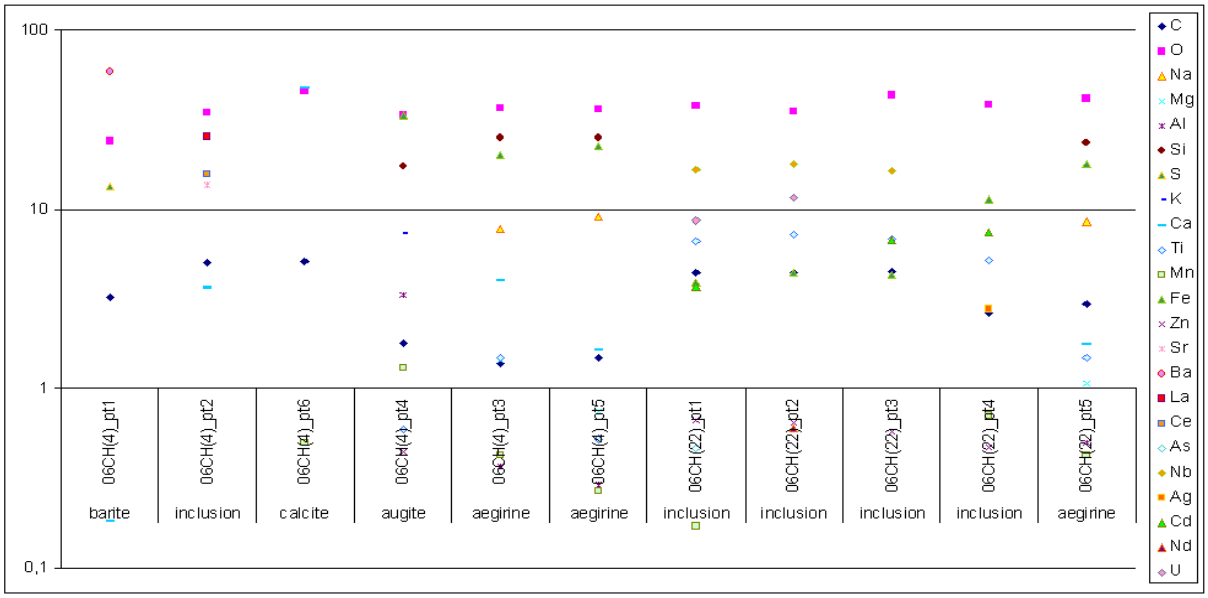

Fig. 28. The results of microanalysis of the REE and U inclusions.

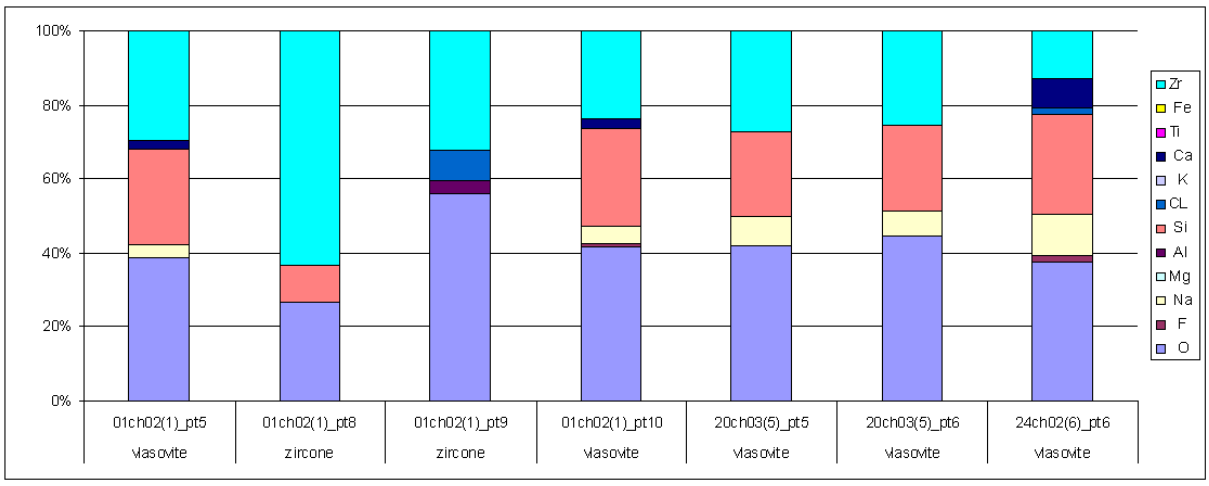

Fig. 29. The results of microanalysis of the zircone and vlasovite.

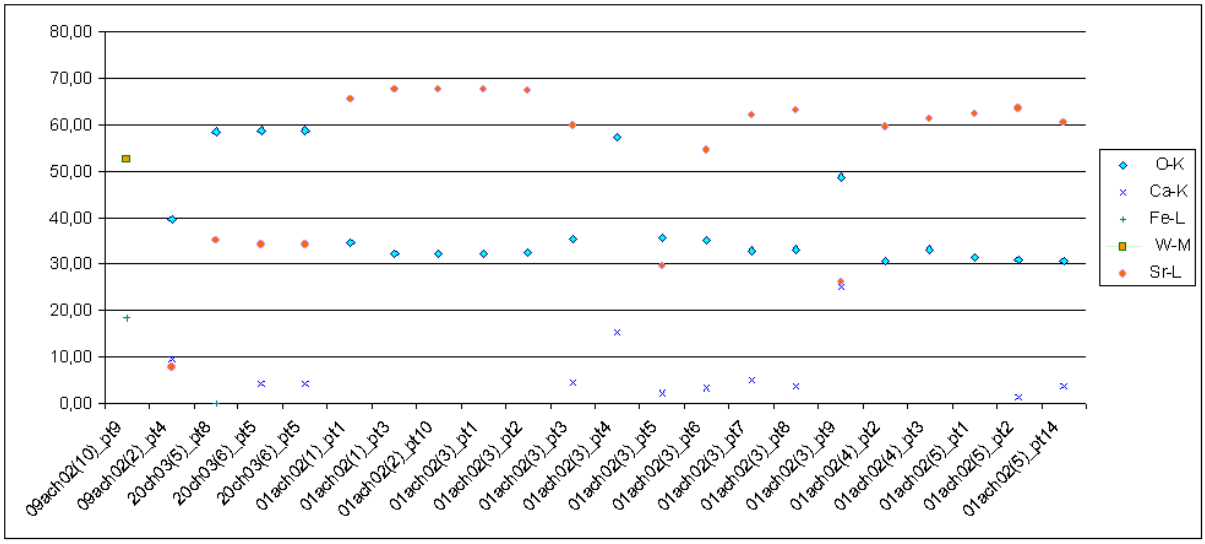

Fig. 30. The results of microanalysis of the Sr inclusions.

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