COMPLEXUL MUZEAL
BISTRIȚA NĂSĂUD

STUDII ȘI CERCETĂRI
Biology

20

BISTRIȚA

2015

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EDITORIAL BOARD OF
Series Biology
Published by the Museum of Bistriţa-Năsăud, Natural Sciences Section
Editorial office: 19 Gen. Grigore Bălan St.
420016 BISTRIŢA
Phone: 004 0263 211063
Fax: 0263 230046

Editura ECOU TRANSILVAN
ISSN 2069 - 1521

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SAMENKEIMUNG, BLATTPROTEIN - UND ASSIMILATIONSPIGMENT-GEHALTE, SOWIE ISOPEROXIDASEN - UND ISOESTERASEN-NACHWEIS BEI *FILIPENDULA ULMARIA* (L.) MAXIM

M. KEUL*, Anca-Livia BUTIUC-KEUL**, Adela HALMAGYI*, Alexandra ŞUTEU***

Zusammenfassung. Bei *Filipendula ulmaria* (L.) Maxim (Echtes Mädesüß) ist die Keimung einige Monate alter Samen unter Laborbedingungen (22-24°C) nur mäßig ausgeprägt (maximal bis etwa 38%) und wird außer von der Keimunterlage (Filterpapier, Gartenerde) insbesondere von der Belichtung der eingequollenen Samen unter Beteiligung des Phytochromsystems beeinflußt. In einer *F. ulmaria*-Population bei Valea Ierii (Rumänische Westgebirge) wurden während der Blütezeit (Juli) biometrische Messungen, Bestimmungen des Blattgehaltes löslicher Proteine und Assimilationspigmente, sowie elektrophoretische Analysen zum Nachweis der Isoperoxidasen und -esterasen durchgeführt. Protein- und Assimilationspigment-Gehalte nehmen von den Grundblättern zu den oberen Stängelblättern allgemein signifikant zu. Die Expression der untersuchten Isoenzyme zeigt praktisch keine genetische Variabilität innerhalb der untersuchten *F. ulmaria*-Population.

Stichwörter: *Filipendula ulmaria*, Samenkeimung, Isoperoxidasen - und Isoesterasen-Muster, Blattprotein - und Assimilationspigment-Gehalte.

Einleitung

Mädesüß-Arten (Gattung *Filipendula*, Fam. *Rosaceae*) sind in der gemäßigten und subarktischen Nordhalbkugel (je nach Autor) mit 12-15 Arten (bzw. 18-20 Taxa) verbreitet (Schanzer, 1994, Lee et al., 2009; Jeelani et al., 2011), wobei in Europa und Rumänien die beiden Arten *Filipendula ulmaria* (L.) Maxim (Echtes Mädesüß) und *F. vulgaris* Moench (Kleines Mädesüß) beheimatet sind (Ciocârlan, 2009). Die Arten der Gattung *Filipendula* und insbesondere das Echte Mädesüß (*F. ulmaria*) sind in letzter Zeit zunehmend eingehenderen Untersuchungen unterworfen worden, die einerseits Fragen ihrer Systematik und Syntaxonomie, Ökologie und Ökophysiologie u.a. zu klären versuchen

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(Grime et al., 1981; Schanzer, 1994; Opitz von Boberfeld et al., 2001; Falińska et al., 2010) und andererseits das Ziel verfolgen, die Erfahrungen der europäischen und asiatischen Volksmedizin wissenschaftlich zu untermauern, um sie in der modernen Phytopharmakologie zu verwerten (Vogl et al., 2013).

Filipendula ulmaria ist in der Volksheilkunde Europas seit uralten Zeiten vornehmlich als entzündungshemmendes, schmerzstillendes und fiebersenkendes Mittel bei Infektionen, Rheuma, Gicht, Grippe, Erkältungen und vielen anderen Leiden verwendet worden (Vogl et al., 2013). Heute steht wissenschaftlich fest, dass diese traditionell überlieferten Heilwirkungen von *F. ulmaria*-Präparaten (Aufgüsse, Tinkturen, Extrakte) sowohl aus Blüten (*Ulmariae flos*) als auch aus Blättern (*Ulmariae folium*) auf ihren Gehalten an Salizylsäure (und ihren Derivaten), Flavonoiden, Gerbstoffen und anderen Wirkstoffen beruhen (Toiu et al., 2011; Denev et al., 2014). Auf dieser Grundlage wurde die Anwendung von *F. ulmaria*-Präparaten vom Europäischen Ausschuss für pflanzliche Arzneimittel (EMA/HMPC, 2011) vorerst bei leichten Erkältungen und Gelenkschmerzen zugelassen. Allerdings zeigen *F. ulmaria*-Präparate nachweislich viele andere, z. T. auch im Tierversuch überprüfte Wirkungen (Vasilauskas et al., 2004; Roman et al., 2008), wie z. B. antibakterielle (Boziaris et al., 2011), antimykotische (Barros et al., 2013), antioxidative und antiulzerogene (Vasilauskas et al., 2004), antitumorale (Lima et al., 2014) und viele andere Eigenschaften, auf die hier nicht weiter eingegangen werden kann (vgl. u.a. die zusammenfassende Darstellung bei Avdeeva und Krasnov (2010).

Im Rahmen eines Forschungsprojektes zur pharmazeutischen Verwertung von *F. ulmaria* wurden u.a. auch vorliegende morphologische und physiologische Untersuchungen über diese Heilpflanze durchgeführt.

Material und Methoden

Untersuchungsmaterial. Verwendet wurden: 1. Samen von *Filipendula ulmaria* (L.) Maxim aus der Samen-Kollektion des Botanischen Gartens „Alexandru Borza" Cluj-Napoca und aus der Wildflora zur Durchführung der Keimversuche; 2. Mädesüß-Exemplare einer blühenden Population (Juli) bei Valea Ierii (Rumänische Westkarpaten) zur Durchführung biometrischer Messungen; 3. frische Blattfragmente blühender Exemplare zur Bestimmung des Blattgehaltes an löslichen Proteinen und Assimilationspigmenten, sowie zum Nachweis der Isoperoxidasen und Isoesterasen.

Untersucht wurden: der zeitliche Ablauf der Samenkeimung im Labor auf Filterpapier und Gartenerde unter verschiedenen Lichtverhältnissen zur Überprüfung der Beteiligung von Phytochromen bei der Induktion der Keimung; Untersuchungen über Wachstum, Entwicklung und Überlebung der aus Samen regenerierten und unter Laborbedingungen weiterkultivierten Keimpflanzen; die quantitative Bewertung einiger morphologischer Merkmale

(Pflanzenhöhe, -masse, Blattgrößen) durch biometrische Messungen an Pflanzenexemplaren aus der natürlichen *Filipendula ulmaria*-Population während der Blütezeit; Bestimmung der Blattgehalte löslicher Proteine und Assimilationspigmente; die Expression der Isoperoxidasen- und Isoesterasen-Muster als Marker für den Nachweis der genetischen Variabilität innerhalb der untersuchten Population.

Die *Keimung der Samen* wurde im Labor in Petri-Schalen auf befeuchtetem Filterpapier (FP) bzw. auf/in einer dünnen Erdschicht (E) bei Zimmertemperatur (22-24°C) unter diffusem Tageslicht und künstlichem Zusatz-Licht (Fluoreszenzröhren, Photonenflussdichte ca. 10^3 µmol/cm^2.s) in 2 Parallelproben von je 20 Samen untersucht. Zum Nachweis der Beteiligung von Phytochromen bei der Keiminduktion wurden Versuche unter Licht (L) und im Dunkeln (D) bzw. unter Weißlicht (WL), Hellrot- (*engl. red= R*) und Dunkelrot-Licht (*engl. far-red= FR*) unternommen. Die entsprechenden Lichtqualitäten wurden mit einer roten Folie für R (maximale Transmission um 660-670 nm) bzw. mit einer Kombination aus einer roten und blauen Folie (maximale Transmission über 720 nm) für FR eingestellt. Die Keimung im Dunkeln erfolgte in einem Dunkelraum bei Auswertung in schwachem Grünlicht.

Der Ablauf der Keimung (in % der ausgesäten Samen) wurde täglich über einen Zeitraum von 36 Tage nach der Aussaat verfolgt. Zur weiteren Beobachtung von Wachstum, Entwicklung und Überlebung wurden die Keimlinge in Blumentöpfe auf Gartenerde verpflanzt und im Labor (22-24°C; Belichtung) weiterkultiviert.

Die Bestimmung des Gesamtgehaltes löslicher Proteine in den Grund- und Stängelblättern wurde bei je drei Pflanzenexemplaren der *F. ulmaria*-Population von Valea Ierii nach Bradford (1976) durchgeführt: je 100 mg der Blattproben wurden kalt in Phosphatpuffer (KH_2PO_4/Na_2HPO_4) bei pH = 6,1 gemörsert, der Extrakt 15 min mit 10.000 rpm bei 4°C zentrifugiert und die Extinktion im Überstand bei 595 nm (Spektrophotometer UV-VIS, Metertech) gemessen. Als Standardprotein wurde Rinderserumalbumin (ASB, Sigma) verwendet.

Die *Bestimmung der* Assimilationspigment-Gehalte in den Grund- und Stängelblättern wurde nach Extraktion in Dimethylformamid (DMF) im Dunkeln bei 4°C durchgeführt (Moran und Porath, 1980). Die Gesamtgehalte an Chlorophyll a (chl *a*), Chlorophyll b (chl *b*) und Carotenoiden (Caroten und Xanthophyll, *car*) wurden spektrophotometrisch (Spektrophotometer UV-VIS, Metertech) bei 664, 647 und 480 nm nach Wellburn (1994) wie folgt bestimmt: chl *a* (mg/g FM) = 11,65 A_{664} – 2,69 A_{647} . v/FM; chl *b* (mg/g FM) = 20,81 A_{647} – 4,53 A_{664} . v/FM; *car* (mg/g FM) = (1000 . A_{480} – 0,89 . chl *a* – 52,02 . chl *b*) /245 . v/FM, wobei A_{664}, A_{647} und A_{480} = Absorbanz der Pigmente bei den

entsprechenden Wellenlängen; V=Volumen des Extraktes (ml); FM= Frischmasse der Gewebeprobe (mg).

Die *elektrophoretische Analyse der Isoperoxidasen und Isoesterasen.* Frisch geerntete Blattfragmente von je 5 *F. ulmaria*-Exemplaren wurden auf Eis mit Hilfe von Glassplittern gemörsert, das Homogenat w/v in Extraktionspuffer (0,1 M Tris-HCl, pH 7,5; 1 mM EDTA; 10 mM $MgCl_2$; 10 mM KCl_2; 14 mM 2-Mercaptoethanol, 10-50 mg/ml festes Polyvinyl-Pyrrolidon -PVP-40) suspendiert (für den Nachweis der Peroxidasen enthielt die Pufferlösung kein 2-Mercaptoethanol), zentrifugiert (10^4 rpm, 10 min, 4 °C) und der Überstand elektrophoretisch analysiert.

Die *Auftrennung* der Isoenzyme erfolgte in einem Polyacrylamid-Gel-System (Elektrophoresegerät Consort[1]) ohne SDS (Nativ-PAGE) durch isoelektrische Fokussierung (IEF) bei 120 V, Laufzeit 1,5 h und Gel-Konzentration 5%. Der Puffer der Migrationsküvetten enthielt 20 mM NaOH/10 mM H_3PO_4, ohne Sammelgel. Das Entwickler-Gel bestand aus Acrylamid/Bis-Acrylamid, Ampholin A pH=3,5-5,0/ Ampholin B, pH=3,5-10,0 (1:1), dest. Wasser, Ammoniumpersulfat und Temed.

Der *spezifische* (*histochemische*) *Nachweis der Isoenzyme* erfolgte mittels einer Kombination mehrerer Rezepturen, die sich in unseren Arbeiten gut bewährt hat (Butiuc-Keul et al., 2007; Keul et al., 2012). Die Peroxidasen erscheinen innerhalb von 30-60 min nach Entwicklung mit 0,05 M Natriumacetat, pH 5,0 (50 ml); 10 mg $CaCl_2$; 3% H_2O_2 (3-Amino-9-Ethyl-Carbazol (50 mg); Dimethylformamid (5 ml) als rotbraune Streifen; zum Nachweis der Esterasen wurden die Gele mit 0,2 M Tris-HCl, pH 7,0 (50-100 ml), 50 mg α-Naphthylacetat; 50 mg β-Naphthylacetat und 50 mg Fast Blue BB bei Zimmertemperatur bis zum Erscheinen verschiedenfarbiger Streifen (rot, braun, schwarz) inkubiert. Die digitalen Fotografien der entwickelten Gele (Olympus-Kamera) wurden mit Paint Shop Pro ausgearbeitet.

Die Endergebnisse der durchgeführten quantitativen Daten wurden als Mittelwerte (m) und Standardabweichungen (s) dargestellt. Als Maß zum Vergleich der Variabilitäten zwischen den einzelnen Proben wurde der Variabilitätskoeffizient v (%) nach K. Pearson (v= s. 100/m) berechnet.

Ergebnisse und Diskussion

Keimversuche. Neuere Untersuchungen über die Keimung von *F. ulmaria*-Samen und über die ökologische Bedeutung der dabei beteiligten Umweltfaktoren wurden insbesondere bei der Lösung praktischer Fragen zur Bewirtschaftung und Renaturierung von Nass- und Feuchtwiesen (Patzelt, 1998; Opitz von Boberfeld et al., 2001; Ludewig et al., 2014) oder für gärtnerische Zwecke (Kootenay Local Agricultural Society, 2008) sowohl unter

[1] Gerätespende der Al. v. Humboldt-Stiftung

Laborbedingungen als auch in Freiland-Versuchen, einschließlich unter Berücksichtigung von Samenbanken (Maas, 1989; Wagner et al., 2003; Hölzl und Otto, 2004) durchgeführt. Die Keimergebnisse der einzelnen Forschungsbeiträge fielen je nach den konkreten Versuchsbedingungen z. T. recht unterschiedlich aus, woraus die Komplexität dieses scheinbar einfachen physiologischen Prozesses dokumentiert wird (vgl. Baskin und Baskin, 2014) und daher Vorsicht bei der Interpretation von Keimergebnissen geboten ist (Grime et al., 1981).

Filipendula ulmaria hat nach Ellenberg eine hohe Feuchtezahl (8) und daher zeigen die Samen einen hohen Wasserbedarf bei der Keimung (Ludewig et al., 2014). Unter Feldbedingungen betragen Keim- und Lebensfähigkeit der *F. ulmaria*-Samen um 50-60% (Ludewig et al., 2014). Im Boden sinkt ihre Keimfähigkeit innerhalb von 2 Jahren bis auf 10% ab (Bekker et al., 1998). Frisch geerntete *F. ulmaria*-Samen befinden sich in einem physiologischen Ruhezustand (Maas, 1989), der durch Licht, Kältevorbehandlung bzw. Wechseltemperaturen gebrochen werden kann (Grime et al., 1981). Nach Opitz von Boberfeld (2001) haben hohe Wechseltemperaturen (20/30°C-Varianten) bei *F. ulmaria* keine oder nur eine schwache Wirkung auf die Keimung, während Kaltlagerung um 5°C und nachfolgender Wechsel zu hoher Keimtemperatur (über 25°C) für die Erzielung guter Keimergebnisse empfohlen werden (Kootenay Local Agricultural Society, 2008).

Grime et al. (1981) untersuchten das Keimverhalten der Samen bei 403 Pflanzenarten und erzielten hohe Keimprozente (97%) bei *Filipendula ulmaria*-Samen unter Belichtung mit grünem Sicherheitslicht (?) und abwechselnden Tagestemperaturen (20/15°C), jedoch signifikant niedrigere Keimwerte (53%) im Dunkeln bei Wechseltemperatur (20/15°C), die schwächsten Ergebnisse (13%) jedoch im Dunkeln bei konstanter Temperatur (20°C). *F. ulmaria* und einige andere Arten zeigen nach Grime et al. (1981) außerdem unerwartete Variationen der Keimprozente in Abhängigkeit von der Lagerdauer. Nach Opitz von Boberfeld et al. (2001) wird die Keimung auch durch die Wirkung der Umweltfaktoren während der Samenreife beeinflusst. Andererseits scheint die Überlebung von *F. ulmaria*-Samen in Diasporenbanken von kurzer Dauer zu sein, denn in Vergrabungsversuchen nimmt ihre Keimfähigkeit innerhalb von 2 Jahren auf maximal 10% ab (Bekker et al., 1998).

In vorliegender Arbeit wurde in einem Versuchsansatz zunächst der Ablauf der Keimung einige Monate alter *F. ulmaria*-Samen ohne vorherige Kaltlagerung oder Stratifikation auf Filterpapier bzw. bei Aussaat auf (unter Belichtung) oder unter eine dünne Erdschicht (also im Dunkeln) untersucht (Abb. 1).

Je nach der Versuchsvariante beginnt die Keimung unter Belichtung auf beiden Keimunterlagen (FP und E) etwa nach 5 Tagen, bei Dunkelheit und Aussaat in die Erde jedoch erst 10 Tagen nach der Aussaat, wobei im Licht

maximal 38% auf FP bzw. auf der Erde kaum 25% erreicht werden. Dagegen wird die Keimung im Dunkeln nur zu etwa 6% induziert, unterbleibt jedoch zur Gänze innerhalb der Versuchszeit von 36 Tagen bei Aussaat unter die Erde.

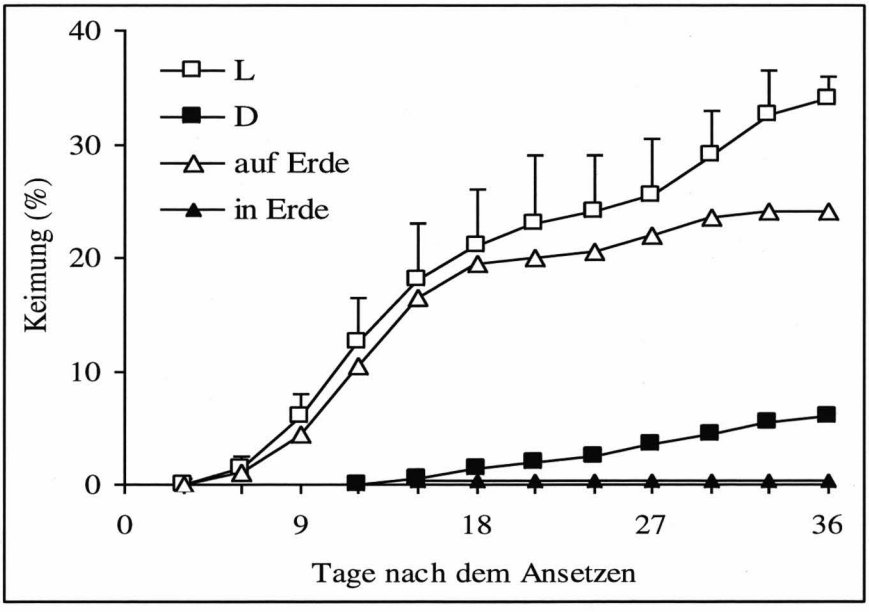

Abb.1. Der Ablauf der Samenkeimung bei *Filipendula ulmaria* auf Filterpapier im Licht (L) und im Dunkeln (D), sowie nach Aussaat auf (L) bzw. unter die Erde (D)

Gegenüber den Befunden anderer Autoren, die bei *F. ulmaria*-Samen nach Kaltlagerung oder bei Wechseltemperaturen unter Belichtung sehr hohe Keimwerte erzielten (z. B. Keimwerte von 98% bei Patzelt, 1998), werden in unseren Versuchen ohne Kältevorbehandlung der Samen unter Belichtung bei konstanter Keimtemperatur (22-24°C) weit kleinere Keimprozente erreicht. Daraus ist die Bedeutung von Kältevorbehandlungen bzw. von Wechseltemperaturen für die Keiminduktion bei *Filipendula ulmaria* ersichtlich, die unter natürlichen Bedingungen unter Einwirkung der Winterkälte erfolgt und bei Temperaturanstieg im Frühling eine rasche Keimung gewährleistet (Maas, 1989; Opitz von Boberfeld et al., 2001).

Die Lichtabhängigkeit der Samenkeimung bei *F. ulmaria* und die Beteiligung des Phytochroms bei der Keiminduktion wurde in einem zweiten Versuchsansatz unter Hellrot (R)- und Dunkelrot (FR)-Belichtung im Vergleich zu einer Weißlicht (WL)- und einer Dunkelkontrolle (D) ohne Kältevorbehandlung der Samen geprüft.

Der Verlauf der Keimung zeigt erwartungsgemäß, dass die besten Ergebnisse (um 23%) im ungefiltertem WL (also höherer Lichtintensität) verzeichnet werden, während die Keimung im Dunkeln (D) dagegen praktisch

ausbleibt. Im Hellrot-Licht (R) unter roter Folie (und demnach niederer Lichtintensität) verläuft die Keimung dagegen langsamer, ist aber nach dem Kurvenverlauf in der geplanten Versuchszeit offensichtlich noch nicht abgeschlossen. Im dunkelroten Licht-Bereich (FR) verläuft die Keimung unter roter+blauer Folie entgegen den Erwartungen zunächst ähnlich wie unter WL, wonach der weitere Ablauf der Keimung nach 15 Tagen bei etwa 15% blockiert wird.

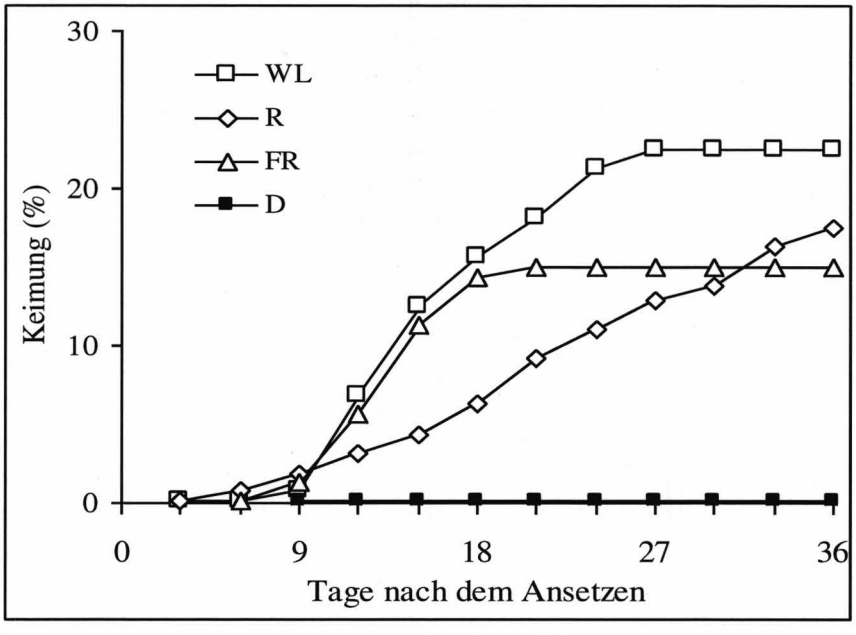

Abb. 2. Der Ablauf der Samenkeimung bei *Filipendula ulmaria* im Dunkeln (D) und nach Belichtung unter Weiß- (WL), Rot- (R=red light, um 660 nm) und Dunkelrot-Licht (FR=far-red light, über 700 nm)

Die erzielten Ergebnisse über die lichtinduzierende Wirkung von WL und R auf die Samenkeimung bei *F. ulmaria* lassen auf die Beteiligung von Phytochromen schließen. Zwar hätte unter FR eine Keimung (wie im Dunkeln) ausbleiben müssen, doch können die scheinbar aus dem Rahmen fallenden Keimergebnisse unter der improvisierten Filterkombination offensichtlich durch die Tatsache etklärt werden, dass die von uns verwendete improvisierte Folienkombination eine (nachgeprüfte) Licht-Transmission z. T. auch im R-Bereich (unter 700 nm) zulassen. Dadurch wird offensichtlich ein Pfr/Ptotal-Verhältnis eingestellt, wodurch die Keimung bei einem Teil der Samen induziert wird. Diese Annahme durch ähnliche Keimverläufe bei *Arabidopsis thaliana*-Samen nach Belichtung zwischen 694 und 700 nm unterstützt, während Dunkelrot-Licht von über 705 nm die Keimung zur Gänze blockiert (Shinomura et al., 1998).

Der theoretische Hintergrund für die über das Phytochromsystem gesteuerte lichtinduzierte Samenkeimung soll hier kurz beleuchtet werden.

Phytochrome (P) sind bei durch Licht kontrollierten Reaktionen (Wachstum, Entwicklung, Keimung) beteiligte Pflanzenpigmente, die in physiologisch aktiven und inaktiven Formen mit unterschiedlichen Absorptionsmaxima vorkommen, die nach spezifischer Lichtabsorption im roten Spektralbereich reversibel ineinander umgewandelt werden: das physiologisch aktive P, mit einem Absorptionsmaximum um 720 nm im DR-Bereich (als Pfr nach *engl.* far red bezeichnet) wird durch Absorption von Lichtquanten dieser Wellenlänge reversibel in die physiologisch inaktive Form (Pr, nach *engl.* red) mit maximaler Absorption im hellroten (HR)-Bereich bei 660 nm umgewandelt. In lichtabhängigen, durch P gesteuerten Prozessen wird unter natürlicher Belichtung ein gewisses Konzentrationsverhältnis zwischen physiologisch aktivem Pfr und physiologisch inaktivem Pr (Pfr/Pr) bzw. zwischen Pfr und der Gesamtmenge (Ptotal) an Phytochrom (Pfr/Ptotal) eingestellt, wodurch diese Vorgänge durch Licht induziert und gesteuert werden (Dechaine et al., 2009).

Wachstum und Entwicklung der Keimpflanzen. Aus Samen regenerierte und in Blumentöpfe auf Gartenerde umgepflanzte *Filipendula ulmaria*-Keimlinge überleben unter Laborbedingungen zu etwa 50%, zeigen aber ein sehr langsames Wachstum und verbleiben lange im Keimblattstadium. Unter natürlichen Bedingungen werden die Pflanzen jährlich vegetativ (klonal) aus dem Wurzelstock regeneriert und erreichen innerhalb von wenigen Monaten die Blüh- und Fruchtreife, was durch Samen in der kurzen Vegetationsperiode bei dieser langsamen Entwicklung wahrscheinlich erst im nächsten Jahr möglich wäre. *F. ulmaria*-Populationen werden demnach aus vegetativ (klonal) und reproduktiv aus Samen regenerierten Pflanzenexemplaren aufgebaut (Falińska et al., 2010).

Unter gemäßigten und subarktischen Klimabedingungen hat die vegetative (klonale) pflanzliche Vermehrung demnach eine entscheidende Bedeutung für die rasche jährliche Neuentwicklung der Vegetation (Aspinwall und Christian, 1992). Die generative Vermehrung durch Samen ist ihrerseits maßgeblich an der Erhaltung der genetischen Diversität in einer klonalen *F. ulmaria*-Population beteiligt (Falińska et al., 2010) und sichert die Ausbreitung der Population und die Besiedlung neuer Areale. Das reproduktive Vermehrungsvermögen wird scheinbar durch das nicht sehr häufige Vorkommen der *F. ulmaria*-Samen in Diasporenbanken (Wagner et al., 2003; Hölzl und Otto, 2004) und durch den raschen Verlust ihrer Lebens- und Keimfähigkeit im Boden (Bekker et al., 1998) negativ beeinflußt.

Biometrische Messungen von je 10-15 Exemplaren der *F. ulmaria*-Population von Valea Ierii (Rumänische Westkarpaten) ergaben während der Blühreife folgende orientierende Werte: Pflanzenhöhe 123,2±11.5 cm, v=9,3%,

Länge des Blütenstandes 8,9±1,9 cm, v=21,3 %; Gesamtmasse 38,0±8,2 g, v=21,6%; Blattlängen: Grundblätter 35,9±7,1 cm; v= 19,8%; mittlere Stängelblätter 22,6±4,5 cm; v=19,9%; obere Stängelblätter 11,9±2,9 cm; v=24,3%, Blätter im Blütenstand 5,5±1,2 cm; v=21,8%.

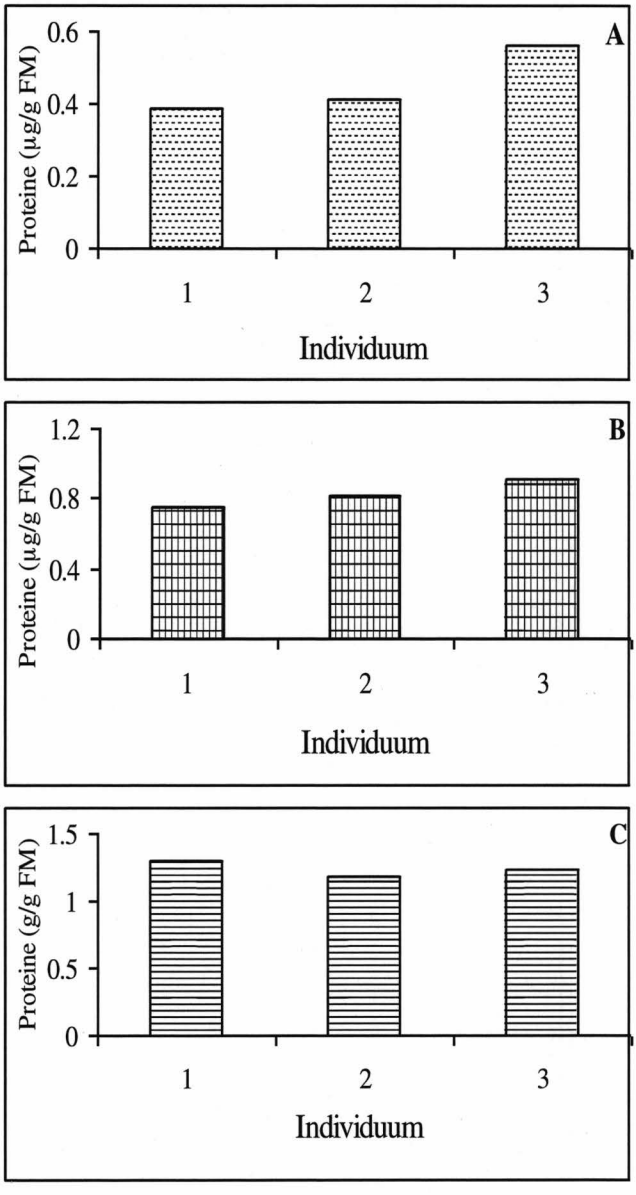

Abb.3. Gesamtgehalte löslicher Proteine der Blätter bei 3 *Filipendula ulmaria*-Exemplaren der Valea Ierii-Population
(A. Grundblätter; B. mittlere und C. obere Stängelblätter)

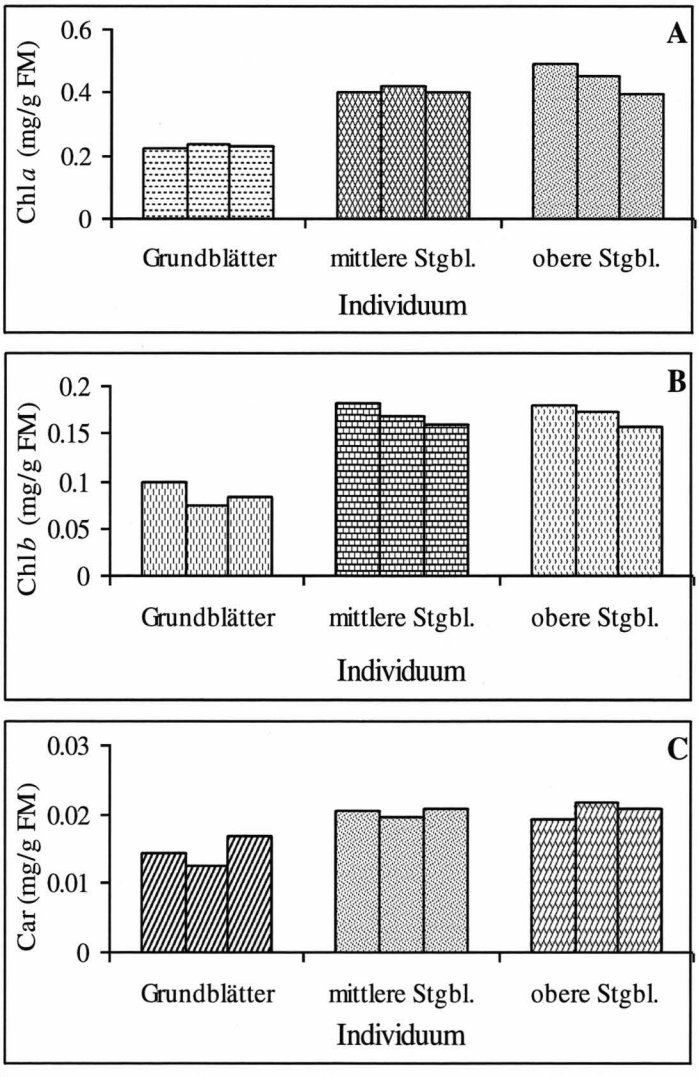

Abb.4. Assimilationspigment-Gehalte der Blätter bei drei untersuchten *Filipendula ulmaria*-Exemplaren der Valea Ierii-Population (A. Grundblätter; B. mittlere; C. obere Stängelblätter)

Die löslichen Protein- (Abb. 3, A-C) und Assimilationspigment-Gehalte (Abb. 4, A-C) der Blätter bei je drei *F. ulmaria*-Exemplaren (=Individuen) zeigen bei zunehmender Insertionshöhe auf dem Stängel mehr oder weniger stark ausgeprägte Differenzen, die am besten anhand der in Tabelle 1 eingetragenen Mittelwerte, Standardabweichungen und Variabilitätskoeffizenten diskutiert werden können.

In den Grundblättern variiert der Blattproteingehalt der untersuchten Individuen zwischen 0,390 und 0,561 µg/g FM, bei einem Mittelwert von 0,455 ± 0,092 µg/g FM und einem Variabiltätskoeffizienten v=20,2%. In den

mittleren Stängelblättern der untersuchten Exemplare nimmt der Proteingehalt zwischen 0,753 und 0,912 µg/g FM (Mittelwert 0,827±0,079, v=9,5%) bis fast auf das Doppelte und in den oberen Stängelblättern bis fast auf das Dreifache (1,186-1,305 µg/g FM, Mittelwert 1,243±0,06, v=4,9%) gegenüber den Grundblättern signifikant zu, wobei die Variation dieses biochemischen Parameters mit der Lage der Blätter auf dem Stängel gleichzeitig abnimmt, d. h. dass die Protein-Blattgehalte bei höherer Lage der Blätter auf dem Stängel konstantere Werte erreichen.

Tabelle 1. Gesamtgehalte an löslichen Proteinen und Assimilationspigmenten (Chlorophyll *a*, *b* und Carotenoide) in Grund- und Stängelblättern bei *Filipendula ulmaria* von Valea Ierii (m=Mittelwert; s=Standardabweichung; v=Variabilitätskoeffizient).

Parameter	Grundblätter	Mittlere Stängelbl.	Obere Stängelbl.
lösl.	0,390	0,753	1,305
Blattproteine	0,415	0,817	1,186
(µg/g FM)	0,561	0,912	1,238
m±s	**0,455±0,092**	**0,827±0,080**	**1,243±0,060**
v	**20,2%**	**9,8%**	**4,8%**
Chlorophyll *a*	0,075	0,403	0,399
(mg/g FM)	0,099	0,421	0,414
	0,116	0,401	0,399
m±s	**0,096±0,020**	**0,408±0,011**	**0,404±0,009**
v	**20,8%**	**2,7%**	**2,2%**
Chlorophyll *b*	0,044	0,161	0,180
(mg/g FM)	0,044	0,188	0,177
	0,053	0,160	0,203
m±s	**0,047±0,010**	**0,170±0,016**	**0,186±0,014**
v	**21,3%**	**9,2%**	**7,5%**
Carotenoide	0,008	0,021	0,023
(mg/g FM)	0,008	0,019	0,022
	0,079	0,021	0,024
m±s	**0,008±0,0003**	**0,020±0,0007**	**0,023±0,001**
v	**3,3%**	**3,6%**	**4,3%**

Die Assimilationspigment-Gehalte (für Chl *a*, *b* und Carotenoide) der Blätter zeigen allgemein viel konstantere Werte zwischen den einzelnen Individuen (Tabelle 1). Dabei werden in den Grundblättern sowohl die kleinsten Gehalte an Chl *a* (0,0751-0,116 mg/g FM; im Mittel 0,096±0,02 mg/g FM; v=20,8%) und Chl *b* (0,044-0,053 mg/g FM; im Mittel 0,047±0,0055 mg/g FM; v=11,7%) als auch die größten Variationskoeffizienten (v) zwischen den einzelnen Werten der untersuchten Individuen festgestellt. In den mittleren und oberen Stängelblättern nehmen alle Pigment-Gehalte (Chl *a*= 0,399-0,421 mg/g

FM; v= 2,2-2,7%; Chl *b*= 0,17-0,186 mg/g FM; v=7,5-9,2%); *Car*= 0,02-0,023 mg/g FM; v=3,6-4,3%) gegenüber den entsprechenden Gehalten in den Grundblättern 2,5-4mal bei gleichzeitiger Abnahme der Variabilität (v) zwischen den analysierten Pflanzenexemplaren zu.

Nachweis von Isoperoxidasen und Isoesterasen. Die auf Polyacrylamid in einem pH-Gradienten von 3 bis 9 durchgeführte elektrophoretische Trennung der Isoperoxidasen und Isoesterasen bei je 5 *F. ulmaria*-Individuen der Population von Valea Ierii (nicht dargestellte Zymogramme) führte zu folgenden Schlussfolgerungen:

Das Isoperoxidasen-Zymogramm zeigt bei allen 5 untersuchten *F. ulmaria*- Exemplaren ein identisches Muster mit 1 neutralen Isoperoxidase entsprechend pH 7 in der Mitte des Migrationsgels und einer schwach sauren Isoperoxidase bei 4 der untersuchten Pflanzenexemplare.

Das Zymogramm-Muster der Isoesterasen ist bei allen untersuchten *F. ulmaria*-Exemplaren identisch ausgeprägt und weist eine Isoesterase im stark basischen (pH 9) und eine zweite Isoesterase im neutralen Bereich (pH 7) auf.

Aufgrund der verwendeten Isoenzym-Marker ist praktisch keine genetische Variabilität in der *F. ulmaria*-Population bei Valea Ierii nachzuweisen. Mit Hilfe anderer Marker konnten Falińska et al. (2010) dagegen eine hohe genetische Diversität in einer klonalen *F. ulmaria*-Population feststellen.

Schlussfolgerungen

Die Keimung einige Monate alter *Filipendula ulmaria*-Samen wurde unter Laborbedingungen (22-24°C) ohne vorherige Kältevorbehandlung auf Filterpapier (FP) und Gartenerde (E) unter Lichteinfluss (L) und im Dunkeln (D) untersucht. Bei Belichtung erreicht die Keimung unter diesen Bedingungen auf FP maximal 38%, auf Gartenerde knapp 25%, während sie im Dunkeln kaum 6% beträgt.

Die Beteiligung des Phytochroms bei der lichtinduzierten Samenkeimung wurde unter Hellrot (R)- und Dunkelrot (FR)-Licht gegenüber einer Weißlicht (WL)- und einer Dunkelkontrolle (D) ohne Kältevorbehandlung der Samen geprüft.

Erwartungsgemäß wurden die besten Keimergebnisse (23%) im ungefiltertem WL (hoher Lichtintensität) verzeichnet, während die Keimung im Dunkeln ausbleibt. Die erzielten Ergebnisse der R- und FR-Wirkung werden besprochen.

Bei den aus Samen regenerierten und im Labor weiterkultivierten *F. ulmaria*-Keimlingen verlaufen Wachstum und Entwickeln bei einer Überlebensrate von unter 50% äußerst langsam.

Zur quantitativen Erfassung morphologischer Merkmale (Pflanzenhöhe, Blütenstand-Länge, Gesamtmasse, Blattgrößen) wurden biometrische

Messungen in der *F. ulmaria*-Population (Valea Ierii, Rumänische Westkarpaten) während der Blütezeit durchgeführt.

Die löslichen Gesamtprotein- und Assimilationspigment-Gehalte (Chlorophyll a und b; Carotenoide) der mittleren und oberen Stängelblätter nehmen bei den 3 untersuchten *F. ulmaria*-Exemplaren von Valea Ierii gegenüber den Grundblättern allgemein signifikant zu.

Die identische Ausprägung der in den Zymogrammen identifizierten Isoperoxidasen und Isoesterasen bei allen untersuchten Exemplaren lässt keine genetische Variabilität innerhalb der *Filipendula ulmaria*-Population bei Valea Ierii erkennen.

Rezumat. La specia oficinală *Filipendula ulmaria* (L.) Maxim (creţuşca) s-au urmărit germinaţia seminţelor în condiţii de laborator, creşterea, dezvoltarea şi supravieţuirea plantulelor regenerate din seminţe şi transplantate în ghivece pe sol, iar la exemplarele de plante recoltate în perioada înfloririi (luna iulie) dintr-o populaţie spontană de *F. ulmaria* identificată la Valea Ierii (M-ţii Apuseni) s-au efectuat măsurători biometrice asupra unor indici morfologici şi s-au prelevat probe de frunze pentru determinarea conţinutului în proteine solubile şi pigmenţi asimilatori, precum şi pentru evidenţierea patternului izoperoxidazelor şi izoesterazelor ca markeri pentru aprecierea variabilităţii genetice în cadrul populaţiei.

Experienţele de germinare s-au efectuat cu seminţe nestratificate, în condiţii de laborator la 22-24°C, la lumină albă şi la întuneric. Rezultatele denotă că germinaţia seminţelor nestratificate la lumină este de maxim 38% pe suport de hârtie de filtru şi de 25% pe sol, comparativ cu doar sub 6% la întuneric. Rezultatele confirmă rolul luminii în inducerea germinaţiei seminţelor la *Filipendula ulmaria*. Date experimentale suplimentare obţinute în lumină spectrală roşie (*red* la 660 nm şi *far red* la 730 nm) întăresc ipoteza privind implicarea fitocromului în germinaţia fotoindusă la această specie.

Creşterea şi dezvoltarea plantulelor de *F. ulmaria* regenerate din seminţe şi cultivate în condiţii de laborator se desfăşoară extrem de lent, gradul de supravieţuire fiind de sub 50%.

Conţinuturile în proteine solubile totale şi în pigmenţi asimilatori (clorofila *a*, *b* şi carotenoizi) din frunzele tulpinale superioare şi mijlocii marchează o creştere semnificativă în perioada înfloririi comparativ cu cele ale frunzelor bazale.

Exprimarea identică a patternului izoperoxidazelor şi izoesterazelor la toate exemplarele de *F. ulmaria* analizate sugerează că variabilitatea genetică apare ca practic inexistentă în populaţia de la Valea Ierii prin utilizarea acestor markeri.

DANK: Die Arbeit wurde vom Ministerium für Erziehung und Forschung Rumäniens durch ein Biostar-Projekt im Rahmen des „Nucleu"-Programmes finanziell unterstützt. Der Alexander von Humboldt-Stiftung danken wir für die Gerätespende zur Durchführung molekularbiologischer Forschungen. Für die Hilfe bei der Beschaffung des pflanzlichen Untersuchungsmaterials sei Herrn Dr. Gheorghe COLDEA,

Korrespondierendes Mitglied der Rumänischen Akademie, auch an dieser Stelle gedankt.

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GENETIC VARIABILITY IN SOME POPULATIONS OF *EPILOBIUM* SPECIES FROM TRANSYLVANIA REVEALED BY ISOENZYME MARKERS

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Abstract. Different populations of 6 species of *Epilobium* from Transylvania (Romania), as *E. hirsutum, E. montanum, E. palustre, E. nutans, E. alsinifolium, E. parviflorum*, and of *Chamerion angustifolium* were studied by isoenzyme markers in order to evaluate their genetic polymorphism. Isoenzymes are useful markers for quick screening in populations in order to select valuable plant species for different biotechnological purposes. The genetic polymorphism revealed by isoperoxidase and isoesterase markers in populations of *Epilobium* species and *Chamerion angustifolium* is extremely low. Genetic polymorphism between populations was observed only in *E. montanum*. The analyzed *Epilobium* species showed only acid isoperoxidases and isoesterases, some of them could ensure the discrimination between species. Discrimination between *E. palustre* and *E. nutans* was not possible by these markers, more enzymatic systems or other molecular markers should be investigated. Individuals of *Chamerion angustifolium* show distinct patterns of isoperoxidases and isoesterases as compared to *Epilobium* species.

Key words: *Epilobium*, isoperoxidases, isoesterases, genetic variability.

Introduction

Epilobium is the largest genus of the plant family *Onagraceae,* with 165 species (185 taxa) worldwide, except Antarctica. *Epilobium* species were used in Central and Eastern Europe, as well as in certain areas of North America, to treat a variety of diseases and enhance wound healing. *Epilobium* has been used as an antispasmodic for conditions such as asthma, and whooping cough. *Epilobium* was also used to treat diarrhea, bowel problems, and colitis. It has been used successfully for bladder health maintenance, male health maintenance, hormonal imbalances, and urinary system health (Deliu et al., 2013).

There are a variety of herbal remedies containing *Epilobium* extracts efficient in treating prostate diseases. Several studies revealed different

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Epilobium species with curative effects in the case of prostatitis, benign tumor, and hypertrophy of the prostate gland. Many authors reported that the condensed tannins showed negligible antitumor activity, while ellagitanins had antitumor activity (Steenkamp et al., 2006, Zhang et al., 2009). Certain species of the genus *Epilobium*, as *E. hirsutum*, have been identified as beneficial in inhibiting the enzymes 5 alpha-reductase and aromatase, enzymes which are involved in the etiology of benign prostatic hyperplasia (Vitalone et al., 2003; Steenkamp et al., 2006).

The active constituents of *Epilobium* species are not well known, but it is considered that they include two pharmacologically important compounds: flavonols (glycosides of myricetin and quercetin) (Bazylko et al., 2007; Hevesi Tóth et al., 2009), and macrocyclic ellagitannins such as oenothein A (OeA) and B (OeB) (Ducrey et al., 1997; Shikov et al., 2010). Additionally, *Epilobium* species synthesize sterols (Pelc et al., 2005; Hevesi Tóth et al., 2009), triterpenes (Nowak and Krzaczek, 1998), gallic, chlorogenic, and ellagic acids (Hevesi Tóth et al., 2009), fatty acids (Pelc et al., 2005), and other compounds such as a mucilage, sugars, vitamins and volatile oils.

For these reasons it is important to investigate the native *Epilobium* species from Romania (Keul, 2013), in order to evaluate their genetic variability in populations, the content of valuable compounds and other. In Transylvania there are about 15 species containing valuable pharmaceutical compounds (Bejenaru et al., 2009; Tămaş et al., 2009), most of them being perennial. Isoenzymes are valuable markers for rapid evaluation of genetic polymorphism in populations and between populations (Hamrick et al., 1991; Gitzendanner and Soltis, 2000).

In this paper several native species of *Epilobium* from Transylvania were studied regarding genetic variability in populations in order to ensure information about the natural germplasm and possible use in biotechnology and pharmacy.

Material and Methods

Isoenzyme analysis in *Epilobium* species

Different populations of 6 species of *Epilobium* and *Chamerion angustifolium* were studied as follows: *E. hirsutum* L.: 5 individuals from Vălişoara, Alba county, and 5 individuals from Şăulia, Mureş county; *E. montanum* L.: 5 individuals from Muntele Mare, Cluj county and 5 individuals from Poiana Horea, Cluj county; *E. palustre* L.: 5 individuals from Şesuri, Maramureş county; *E. nutans* F.W. Schmidt: 5 individuals from Muntele Mare, Cluj county; *E. alsinifolium* Vill.: 5 individuals from Muntele Mare, Cluj county; *E. parviflorum* Schreb.: 5 individuals from Cluj-Napoca, Cluj county and *Chamerion angustifolium* (L.) Holub: 5 individuals from Arieşeni, Alba county.

Two enzyme systems have been studied in all samples: peroxidases (E.C.-No. 1.11.1.7; *Per*), and esterases (E.C.-No. 3.1.1.1; *Est*). Both enzyme systems have been separated on polyacrylamide gels by isoelectric focusing (IEF) (Acquaah, 1992). Gel concentration was 5%. For gel preparation a stock solution of acrylamide/bisacrylamide mixed with ampholine A, pH=3.5-5.0/ampholine B pH=3.5-10.0 ratio 1:1, H_2O, ammonium persulphate 10% and Temed were used. Two buffers were used in cuvettes: 20 mM NaOH/10 mM H_3PO_4; running was performed one hour at 120 V with a Consort device. Histochemical identification of enzyme was performed according to several protocols described elsewhere (Acquaah, 1992).

Genetic similarities between the *Epilobium* species were measured by the Jaccard's similarity coefficient (Jaccard, 1908) with the Past programme and the generated similarity coefficients were used for constructing a dendrogram with UPGMA option using the same programme.

Results and Discussions

Separation of isoenzymes by isoelectric focusing allow identification of different alkaline, neutral or acid isoforms. By ampholine adition in the acrylamide gel, a pH gradient from 9 (in the upper part of the gel) to 3 (in the bottom of the gel) was obtained. In *Epilobium* and *Chamaerion* species only acid isoperoxidases and isoesterases were identified.

Izoenzymes distribution in different populations of *Epilobium* and *Chamaerion* species from Transylvania is shown in Table 1 and the number of izoenzymes/locus, in Table 2.

Table 1. Izoenzymes distribution in different populations of *Epilobium* and *Chamerion* species from Transylvania (P=population, AB=Alba; CJ=Cluj; MM=Maramureş; *Per-C1-4*=acid isoperoxidases; *Est-C1-6*=acid isoesterases; + presence in all individuals; - absence in all individuals; +,- presence/absence in some individuals

Species/ Populations		Izoenzymes									
		Isoperoxidases Per-				Isoesterases *Est-*					
		C1	*C2*	*C3*	*C4*	*C1*	*C2*	*C3*	*C4*	*C5*	*C6*
Epilobium hirsutum	Vălişoara (AB)	+	+	-	+,-	+	+	+	+	-	-
	Şăulia (MS)	+	+	-	+,-	+	+	+	+	-	-
E. montanum	Muntele Mare (CJ)	+	+	+,-	+,-	+	+,-	+	-	-	-
	Poiana Horea (CJ)	+	+	+,-	+	+	+,-	-	-	-	-
E. palustre	Şesuri (MM)	+	+	+,-	-	+	+	+,-	+,-	-	-
E. nutans	Muntele Mare (CJ)	+	+	+,-	-	+	+	+,-	+,-	-	-

E. alsinifolium	Muntele Mare (CJ)	+	+	+	-	+	+	+	+,-	+,-	-
E. parviflorum	Cluj-Napoca (CJ)	+	+	-	+	+	+	-	-	-	-
E. parviflorum	Cluj-Napoca (CJ)	+	+	-	+	+	+	-	-	-	-
Chamerion angustifolium	Arieşeni (AB)	+	+	+	+	+	+	-	-	-	+

Table 2. Number of izoenzymes/locus in different populations of Epilobium and Chamaerion species from Transylvania (P=population, AB=Alba; CJ=Cluj; MM= Maramureş; Per-A=alkaline peroxidases; Per-B=neutral peroxidases; Per-C=acid peroxidases; Est-A=alkaline esterases; Est-B=neutral esterases; Est-C=acid esterases)

Species/ Populations		No. isoenzyme/locus					
		Per-A	*Per-B*	*Per-C*	*Est-A*	*Est-B*	*Est-C*
E. hirsutum	P1: Vălişoara (AB)	-	-	2-3	-	-	4
	P2: Şăulia (MS)	-	-	2-3	-	-	4
E. montanum	P1: Muntele Mare (CJ)	-	-	2-4	-	-	1-2
	P2: Poiana Horea (CJ)	-	-	3-4	-	-	1-3
E. palustre	Şesuri (MM)	-	-	2-3	-	-	3-4
E. nutans	Muntele Mare (CJ)	-	-	2-3	-	1	3-4
E. alsinifolium	Muntele Mare (CJ)	-	-	3	-	-	3-5
E. parviflorum	Cluj-Napoca (CJ)	-	-	3	-	-	2
Chamerion angustifolium	Arieşeni (AB)	-	-	4	-	-	3

Isoperoxidases analysis

The analysis of peroxidases in *E. hirsutum* showed 3 acid isoperoxidases. The first 2 isoperoxidases are present in all individuals independent of the population, whereas the third acid isoperoxidase is present only in some individuals belonging to Şăulia population (Table 1, 2).

Despite of this, in *E. montanum* the isoperoxidase pattern is different, 4 isoperoxidases were observed in this species (Table 2). The first 2 acid isoperoxidases identified in *E. hirsutum* are also present in *E. montanum* as well, but another acid isoperoxidase appears in some individuals independent of the population. The last acid isoperoxidase observed in *E. hirsutum* is also present in this species. Thus, the polymorphism of isoperoxidases in the individuals belonging to these different populations is extremely low. Peroxidase activity is lower in the individuals belonging to *E. montanum* from

Muntele Mare population despite of those from Poiana Horea population. The acid isoperoxidases 2 and 3 were not observed in the individuals belonging to Muntele Mare population. The genetic polymorphism in populations of *E. montanum* revealed by isoperoxidases is extremely low, but there are several differences between the two populations (Table 1).

Very interesting patterns were observed in *E. palustre* and *E. nutans*. The patterns of the 3 observed isoperoxidases are identical in all examined individuals of these species (Table 2). The first 2 acid isoperoxidases observed in *E. hirsutum* and *E. montanum* are also present in these two species, and the third acid isoperoxidase which is present also in *E. montanum.* For discrimination of *E. palustre* and *E. nutans,* more enzyme systems or other molecular markers should be investigated. The pattern of isoperoxidases is identical in these two species, no genetic polymorphism being observed (Table1).

In *E. alsinifolium* species, 3 acid isoperoxidases were identified, all of them being present in all individuals, no genetic polymorphism was observed (Table 1).

The individuals belonging to *E. parviflorum* species also show 3 acid isoperoxidases in all individuals (Table 1, 2).

In *Chamerion angustifolium*, the same 3 acid isoperoxidases identified in *E. parviflorum* were also present and another acid izoperoroxidase was identified, which is not present in any other *Epilobium* species. The pattern of isoperoxidases is the same in all individuals (Table 1, 2).

Isoesterases analysis

In the individuals belonging to *E. hirsutum* 4 acid isoesterases were identified. All of these isoesterases were present in all individuals independent of the population, the genetic polymorphism could not be observed by these markers (Table 1, 2).

In *E. montanum* individuals, 3 acid isoesterases were observed (Table 2). The first isoesterase is present in all individuals independent of the population, the second one is present only in some individuals independent of the population and the third is present only in the individuals from Muntele Mare population. Thus, in *E. montanum,* genetic polymorphism in the populations and between populations could be observed by isoesterases (Table 1).

Similar situation as in case of isoperoxidase was observed in isoesterases patterns of *E. palustre* and *E. nutans*. The isoesterase patterns are very similar in the individuals belonging to these two species, 4 isoesterases being identified. The isoesterases 3 and 4 are observed only in some individuals belonging to these two species. The discrimination of these two species was not possible by isoeesterases patterns (Table 1, 2).

The isoesterases pattern of *E. alsinifolium* species is different from the patterns of the other *Epilobium* species. Thus, 5 different isoesterases were

observed (Table 2). The first 3 isoesterases are present in all individuals, but the last 2 isoesterases are present only in some individuals, low genetic polymorphism could be revealed by these 2 acid isoesterases (Table 1).

The individuals of *E. parviflorum* species show the first 2 isoesterases also present in *E. alsinifolium*, their presence being constant in all individuals, thus the genetic polymorphism could be not observed (Table 1, 2).

Despite of isoesterases patterns *Epilobium* species, the individuals belonging to *Chamerion angustifolium* show a different isoesterases pattern. The first 2 acid isoesterases present in most of the *Epilobium* species are also observed in *Chamerion angustifolium*, but a distinct acid isoesterase is observed, that is not present in *Epilobium* species (Table 1, 2).

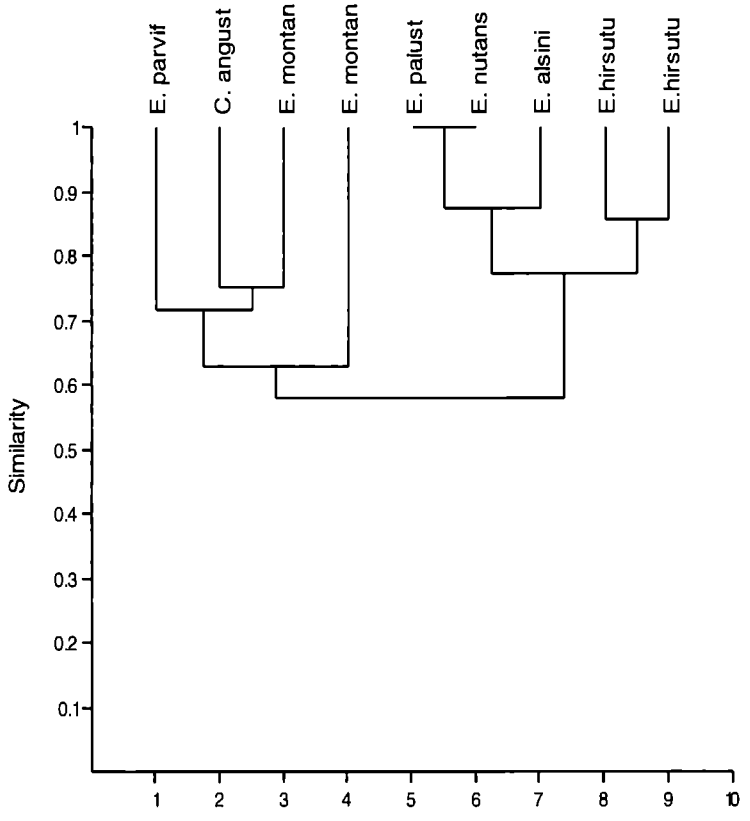

Fig. 1. Dendrogram illustrating similarities among *Epilobium* species and *Chamerion angustifolium* from natural habitats of Transylvania by the UPGMA cluster analysis calculated by isoenzyme markers

Isoenzyme markers were used for calculation of genetic similarities between species from natural habitats. Jacquards coefficient between species ranged from 0.63-1.0 (Fig. 1), but similarities could also be observed form individuals belonging to the same populations as for example *E. hirsutum.* The populations of *E. montanum* are more different then *E. hirsutum* as it could be

observed from dendrogram. The population from Poiana Horea (P2) is closed to population of *Chamerion angustifolium*. The *Epilobium* species are clustered in 3 groups. One group includes the populations of *E. hirsutum* that are almost identical, Jacquards coefficient being 0.85. Another group includes *E. palustre, E. nutans* (Jaccard coefficient being 1.0) and *E. alsinifolium* (Jacquards coefficient being 0.87). The third group includes the populations of *E. montanum, Chamerion angustifolium* (Jaccards coefficient of 0.76) and *E. parviflorum* (Jaccards coefficient of 0.72).

The family *Onagraceae* includes two subfamilies, *Ludwigioideae* (Ludwigia) and *Onagroideae*. The latter comprises six tribes: *Hauyeae, Circaeeae, Lopezieae, Gongylocarpeae, Epilobieae, and Onagreae* (Wagner et al., 2007). The tribe *Epilobiae* includes two genera, *Chamerion* and *Epilobium*, with north temperate origin (Levin et al., 2004). There were many morphological, phytochemical, and genetical analyses performed to give convincing arguments for treating *Chamerion* as a separate genus from *Epilobium* (Strgulc Krajšek et al., 2009). However, authors still use in their works *Epilobium angustifolium* L. instead of the correct denomination of *Chamerion angustifolium* (L.) Holub.

Epilobium genus is remarkable for its morphological, ecological, and cytological diversity (Baum et al., 1994). Taxonomically, *Epilobium* is a very difficult group because of species similarity and frequent interspecific hybridization (Bleeker et al., 2007). Our results confirm this data, by isoperoxidases and isoesterases it is difficult to discriminate close related species, as it was observed in case of *E. palustre* and *E. nutans* that showed the same pattern of these isoenzymes. The isoenzymes could be useful for rapid evaluation of genetic polymorphism in populations, because the analyse is quick and cheap. The results could be used for quick screening in populations in order to select valuable plant species for different biotechnological purposes and for *in vitro* culture and compound biosynthesis.

Conclusions

The genetic polymorphism in the populations of *Epilobium* and *Chamerion* species is extremely low as revealed by isoperoxidases and isoesterases markers.

Low genetic polymorphism was observed in populations of *E. montanum, E. palustre, E. nutans,* and *E. alsinifolium*.

Genetic polymorphism between populations was observed only in *E. montanum*.

The *Epilobium* species showed only acid isoperoxidases and isoesterases, some of them could ensure the discrimination between species.

By isoperoxidase and isoesterase patterns the discrimination between *E. palustre* and *E. nutans* species was not possible.

Individuals belonging to *Chamerion angustifolium* show distinct patterns of isoperoxidases and isoesterases from the individuals belonging to *Epilobium* species, even some of the isoenzymes are shared by these two plant species.

Rezumat. În această lucrare s-au analizat diferite populaţii aparţinând la 6 specii de *Epilobium* din Transilvania, România, cum sunt *E. hirsutum, E. montanum, E. palustre, E. nutans, E. alsinifolium, E. parviflorum* şi *Chamerion angustifolium*, cu ajutorul markerilor izoenzimatici. Aceşti markeri s-au utilizat pentru evaluarea polimorfismului genetic în populaţiile acestor specii. Izoenzimele sunt markeri utili pentru screeningul rapid în populaţii în vederea selecţiei plantelor valoroase cu aplicaţii diverse în biotehnologii. Polimorfismul genetic relevat de izoperoxidaze şi izoesteraze în populaţiile diferitelor specii de *Epilobium* şi *Chamerion* este extrem de redus. Polimorfism genetic interpopulaţional a fost evidenţiat doar la *E. montanum*. S-a observat că patternul izoenzimelor evidenţiate la speciile de *Epilobium* şi *Chamerion* cuprinde numai izoenzime acide, unele dintre ele fiind utile pentru discriminarea speciilor, cu excepţia speciilor *E. palustre* şi *E. nutans*, care au evidenţiat patternuri identice, pentru a căror discriminare este nevoie de investigarea altor sisteme enzimatice, sau utilizarea altor markeri moleculari. Indivizii aparţinând speciei *Chamerion angustifolium* prezintă patternuri ale izoperoxidazelor şi izoesterazelor distincte de cele ale speciilor de *Epilobium* studiate.

ACKNOWLEDGEMENTS: This work was supported from the CEEX-BIOTECH Programme, Project No. 98/01.08.2006, financed by the Romanian Ministry of Education and Research.

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EFFECTS OF *LAMIUM ALBUM* AND *LAMIUM PURPUREUM* EXTRACTS ADMINISTRATION ON THE LIVER FUNCTION IN ANAKINETIC STRESS CONDITIONS

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Abstract. The aim of our study was focused on the analysis of the protector potential of two different hydro-alcoholic extracts of *Lamium album* L. and *Lamium purpureum* L. on some biochemical blood parameters: the total proteins, glucose, cholesterol, and triglicerides levels, as well as the hepatic enzymes TGO and TGP, relating to impairment of liver function in white Wistar rats by anakinetic stress induced during 2 h for 17 days. Animals were treated with 1:1 hydro-alcoholic extract of *L. album and L. purpureum* L (20 mg d.s/100 g bw., for each extract). Extracts were administered for 17 days in white female Wistar rats, weighing 165g ± 20 g before restraining. We found that single administration of vegetable extract of white or red nettle did not cause toxic effects, while treating animals with both kind of nettle extract had beneficial effects of protecting and preventing at almost all levels of the parameters taken into study, in terms of anakinetic stress conditions.

Key words: anakinetic stress, *Lamium album* and *Lamium purpureum* extract, rats, liver.

Introduction

As a general concept, stress is the body's individualized response to external or internal challenges, being an important adverse factor affecting a broad range of human populations. By its effects, stress may cause the etiology of many diseases, such as - cardiovascular disease, diabetes, cancer etc. In response to stressors in the body, a series of behavioral changes occur, of neurochemical and immunological nature, which in conditions of prolonged stress increases the body's vulnerability to the incidence of various diseases. Between various stressful agents, immobilization stress or prolonged anakinetic stress induces reactive oxygen species (ROS) formation, hydrogen peroxide and other reactive species of free radicals, which cause lipid peroxidation, especially at the level of cell membranes with destructive effects on the entire tissue.

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The prolonged anakinetic stress also induces increased levels of glucocorticoid hormones accompanied by increase of serum cholesterol and lipoproteins - risk active factors in the pathology of cardiovascular diseases and diabetes (Berghian et al., 2011; Nayanatara et al., 2012). The presence and the harmful effects of free radicals imposed finding some remedies. These remedies included some extracts or biopreparates of plants. Last decades researchers have identified substances with antioxidant effects that can neutralize or mitigate the harmful effects of free radicals. Polyphenols belong to this category, too. Herbs and other plants that contain more polyphenols, natural antioxidants, is a category of plants sought because of their possible use in phytotherapy. As we know, antioxidants can delay, inhibit or prevent oxidation of substrates oxidized by free radicals cleaning and reducing oxidative stress. However, in disease conditions, defense against ROS is weakened or damaged and oxidant load increases. In those circumstances, the external antioxidants supplementation is essential to counteract the harmful consequences of oxidative stress (Ratnam et al., 2006; Reuter et al., 2010). It is assumed that polyphenols can act as antioxidants with a number of potential mechanisms. Cleaning of free radicals, in which polyphenols can break the free radicals chain reaction as well as the suppression of the free radicals formation through the mechanism of enzyme activity regulation or metal ions chelating involved in the production of free radicals has been reported as the most important mechanism of their antioxidant activity. The interaction between polyphenolic compounds with other physiological antioxidants is another possible antioxidant route for these compounds (Fraga et al., 2010; Perron and Brumaghim, 2009).

In Romania there is an ancient folk tradition regarding the use of some plants as remedies against some diseases. The use of medicinal plants in treating or preventing of various inconveniences aroused interest in folk medicine practices, particularly those transcendental over generations. *Lamium album* L. (white dead nettle) is a medicinal plant widely used in folk and traditional medicine as a powerful astringent and anti-haemorrhagic agent in the reproductive tract (Yalcin and Kaya, 2006). Floral extract (flos) is used as an expectorant and to treat certain respiratory affections, especially catarrh and bronchitis (Bartram, 1998; Blumenthal, 1998; Van Wyk and Wink, 2004). In addition to the anti-inflammatory effect, some of the active components of *Lamium album*, are bacteriostatic and antispasmodic (Paduch et al., 2008). These therapeutic effects are due to a great variety of biologically active components of the plant, which are not yet well studied. Studies carried out on a wide variety of medicinal plants have shown that anti-inflammatory active ingredients in water extracts, include many natural chemical substances such as phenol, alkaloids, glycosides and carbohydrates.

Most likely, *Lamium album* tannins are responsible for the positive effects in gynecological use; saponins give the benefits of tea and other clinical effects of herbal extracts are due to a large variety of essential oils and glycosides (Talhouk et al., 2007). However, mechanism of action and the elements involved in these effects have not been clearly identified.

The aim of our study was to emphasize the protector potential of both *Lamium album* and *Lamium purpureum* hidroalcoholic extracts on some liver morphological and biochemical parameters, in white Wistar rats affected by anakinetic stress condition.

Material and Methods

Experiments were performed on white female Wistar rats, weighing 165 ± 20 g during 17 days. Animals were divided into 6 groups of 6 animals each, as follows: control group (C); anakinetic stressed group (S). The immobilization stress was induced in rats by putting them in 20 cm × 7 cm plastic tubes for 2h/day for 17 days (Marcilhac et al., 1998; Yokus et al., 2005). There are several 3 mm holes at the far end of the tubes for breathing, that allows ample air but animals will be unable to move. Moreover, animals were kept in dark condition in the period of immobilization to emphasize the stress state; *Lamium album* hidroalcoholic extract treated group (LA), which received 20 mg extract/100 g bw, *á jeun*; *Lamium purpureum* hidroalcoholic extract treated group (LP), which received 20 mg extract/100 g bw, *á jeun*; anakinetic stress + LA treated group (SLA) and anakinetic stress + LP treated group (SLP), in the same conditions like previous groups

Animals were obtained from the biobasis of „Iuliu Haţieganu" Medicine and Pharmacy University, Cluj-Napoca and kept under standardized zoohigienical conditions: in accordance to the European Communities Council Directive 2010/63/UE Directive of European Parliament and according to the approval of the Ethics Committee and Animal Protection for Experiments from the Institute of Biological Research, NIRDBS branch, Cluj-Napoca, Romania (Decision 1/28.02.2013).

The hydroalcoholic extracts of *Lamium album* and *Lamium purpureum* was obtained at the "Stejarul" Biological Research Center, Piatra Neamţ. The alcoholic extract (1:1) was obtained with alcohol 70^0. Qualitative analysis of the polyphenolic compounds of the *Lamium album* and *Lamium purpureum* extract was done by thin-layer chromatography (TLC), spectrofotometry and high performance liquid chromatography (HPLC).

Treatment lasted for 17 days. In the 18^{th} day, animals were killed by decapitation after a pre-anesthesia with ether. Fragments of organs were removed and fixed in Bouin liquid fixative and prepared for histology. Staining

was made by haematoxilin-eosin method for histological structure of liver (Mureşan et al., 1974).

The blood was collected and then processed according to analyzed functional parameters. We measured total proteins, glucose, cholesterol and triglycerides. Thus, blood samples were immediately centrifuged, serum harvested and then frozen in Eppendorf vials. Measurements were made with biochemistry semiautomatic analyzer screen point type, with reagents - STATE - FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd Ramsey Minnesota, USA 55303.

Transaminase level was also measured - TGO and TGP (Reitmann and Frankel, 1957).

The biochemical data was statistically processed by means of Student's „t" test. Aberrant values were eliminated by means of Chauvenet's criterion. A probability value of $p \leq 0.05$ was considered.

Results and discussions

Histological study of the liver

Histological study of the liver in group M revealed the characteristic normal structure of the liver parenchyma structure, respectively of hepatic lobe classic structure.

The body of the liver lobule is composed of hepatocytes cords separated by sinusoidal, capillary, bile ducts and supporting tissue. Remack's cell cords have a radial position, converging toward centrilobular vein, these being separated by sinusoidal capillary that present discontinuous endothelium.

Between liver cords and sinusoidal capillaries there is a perisinusoidal space - Disse space. Some kind of "fat storing" mesenchymal cells, responsible for fat liver storing can be found in this space.

Sinusoidal capillaries have basal membrane and discontinuous endothelium being composed of endothelial and Kupffer cells (stellate macrophages). Intralobular supporting tissue is composed of small quantity of connective tissue, collagen and reticulin fibers, probably from Disse spaces. The fine reticular connective tissue supports and also separates hepatocytes of liver cell cords (Figure 1).

Exposure to anakinetic stress of animals in group S caused some changes of liver parenchyma structure, characterized by inducing of a clear moderate dystrophies of hepatocytes, aspect associated with lumen dilation of sinusoidal capillaries surrounding the hepatocyte cords. Some hepatocyte nuclei are irregularly shaped, have low volume, showing picnotic trends (Figure 2).

Both in LA and LP groups the histologic aspect of liver is very close to the group C (Figures 3 and 4).

Histological examination of liver sections in SLA and SLP groups exposed to anakinetic stress and treated concomitantly with those two plant

extracts of *Lamium album,* respectively, *Lamium purpureum*, also revealed no significant morphological changes of hepatic parenchyma compared to group C (Figures 5 and 6).

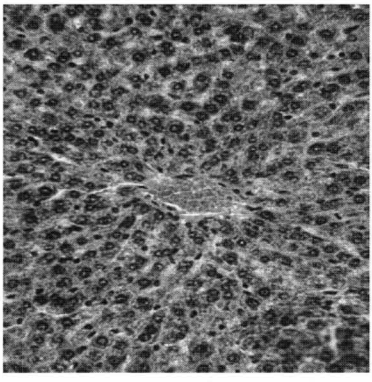

Fig. 1. Normal structurally aspect of liver parenchyma in group C (400 x)

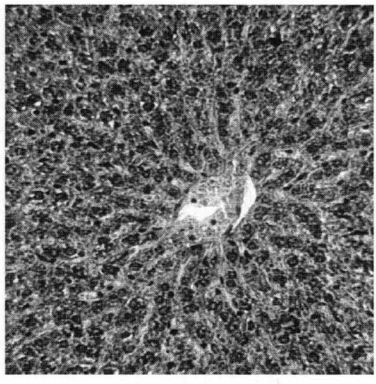

Fig. 2. Group S - Clear dystrophy aspects of hepatocytes whose nucleus presents picnotic trends (400 x)

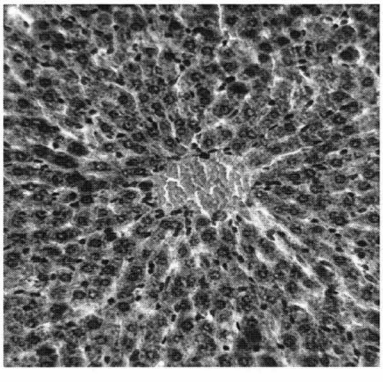

Fig. 3. Aspect close to normal of liver parenchyma in group LA (400 x)

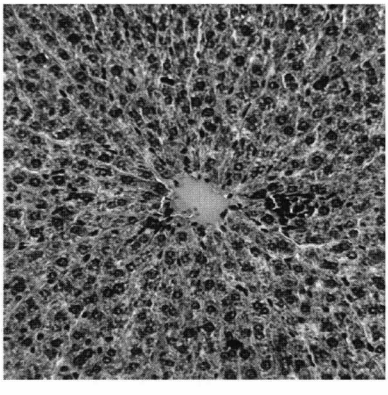

Fig. 4. Aspect close to normal of liver parenchyma in group LP (400 x)

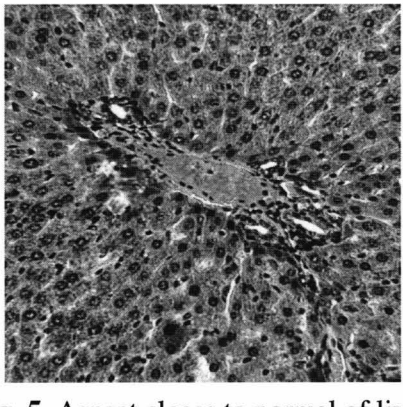

Fig. 5. Aspect closes to normal of liver parenchyma in group SLA (400 x)

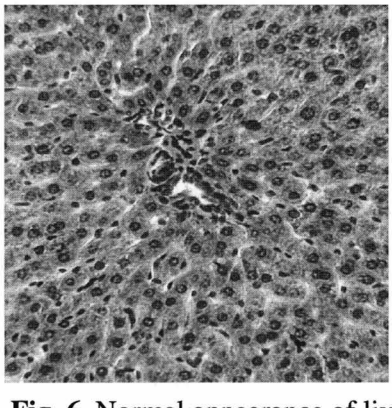

Fig. 6. Normal appearance of liver morphology in group SLP (400 x)

The results of biochemical analysis

The obtained results from glucose levels, serum cholesterol, total protein, triglycerides and serum transaminases (GOT and GPT), are shown in Tables 1, 2. After the applied treatments, it is found that no significant changes in value occur in glucose and total protein level (PT).

Serum cholesterol levels increase significantly (+ 24.73%, p = at limit) in stressed group, slightly decreased in the group treated with white dead nettle extract (-8.32%, p>0.5) and significantly lower in red nettle treated group (-41,60%, p<0,5) than the control group, and also significantly decrease (-55.47%, p<0.001) in SLA group and (-43,50%, p<0,01) in SLP group. Triglyceride concentration is increased in the five experimental treated groups, with 123.95% (p = limit) in group S, 123.16% (p = limit) in group LA, 111.35% (p<0.05) in SLA group, 72,49% (p = limit) in LP group and with 100,08% (p<0,05) in SLP group.

In the tables are shown the mean ± standard error, n = no. individuals / group, p - significantly from 0.05 percent difference from control (D%).

Table 1. The blood glucose level, TP, cholesterol and triglycerides

	M	S	LA	SLA	LP	SLP
Glucose (mg/dL)						
x±ES	138,2±2,65	146,0±5,13	155,16±8,48	144,83±3,27	144,0±6,01	137,8±3,97
D%	-	+5,64%	+12,27%	+4,79%	+12,27%	-0,28%
T.P. (g/dL)						
x±ES	5,233±0,21	5,52±0,276	5,59±0,158	5,28±0,032	5,72±0,26	5,63±0,14
D%	-	+5,54%	+6,88%	+0,39%	+6,88%	+7,66%
Cholesterol (mg%)						
x±ES	181,9±22,1	226,9±6,3	166,8±16,2	81,02±8,55	106,2±9,1	102,8±42,2
D%	-	**+24,73%**	-8,32%	**-55,47%**	**-41,60%**	**-43,50%**
Triglycerides (mg/dL)						
x±ES	24,06±7,22	53,88±16,8	53,69±14,51	50,85±5,16	41,5±3,82	48,14±3,9
D%	-	**+123,95%**	**+123,16%**	**+111,35%**	**+72,49%**	**+100,08%**

Serum aminotransferases are significant changed in TGO whose value increases significantly compared to control group for 4 between treated groups, respectively with 24.75% (p <0.01) in group S, 19.96% (p <0,05) in group LA, 64.48% (p<0.001) in the group SLA, increases by 15.38% (p - NS) in group LP, and, respectively with 25.00% (p<0.01) in group SLP.

TGP level does not suffer major changes in value, compared to controls, only in the SLA group it increases significantly, with 49.65% (p <0.001).

Table 2. TGP and TGO levels

	M	S	LA	SLA	LP	SLP
TGO (µg pyr/ml/h)						
x±ES	143,78±4,4	179,3±8,05	172,48±8,93	236,5±21,45	165±14,205	179,72±7,6
D%	-	**+24,75%**	**+19,96%**	**+64,48%**	+15,38%	**+25,00%**
TGP (µg pyr/ml/h)						
x±ES	282,24±9,7	321,1±17,2	274,8±47,04	422,4±22,51	254,5±18,9	316,8±166
D%	-	+13,77%	-2,63%	**+49,65%**	-9,81%	+12,24%

Analysis of histological study

Histological examination of the liver revealed no significant structural morphological changes of the liver parenchyma in the six experimental groups compared with controls. Histological aspects of the liver morphology, illustrated by hepatocyte moderate, clear dystrophy, associated with lumen dilation of sinusoidal capillaries, observed in group S, exposed to anakinetic stress, do not have a pathological character, changes being reversible, temporary. Clear, moderate dystrophy of hepatocytes, that occurs following to stress exposure (group S), modifications which are the expression of structure injury of endoplasmic reticulum and mitochondria (Lieber, 2000), are not so pernicious, having a temporary, reversible character (Michell and Contan, 2003).

Our results are in agreement with the data obtained by Kumar et al., 2012, who described the induction of morphological and biochemical changes of the liver and kidney structure and functions in laboratory mice, according to the animal exposure to the different conditions of stress .

Lamium album, respectively, *Lamium purpureum* (LA group and LP group) extract plant administration had no adverse effect on the liver structure, the liver generally histology aspect is very close to the group C. The obtained data show that the plant extracts obtained from the two Lamium species do not show toxic action, negative on the structure of the rat liver. We note that in these experimental groups there were increases particularly of TGO levels that, however, are not associated with changes in liver parenchyma structure.

Examination of histological liver sections in SLA and SLP groups also revealed no significant morphological changes of hepatic parenchyma compared to group C. These observations suggest a protective effect of the two plant extracts in the animal exposure to stress conditions. Reports in the literature have shown that plant extracts obtained from several species of Lamium, including those of *Lamium album* and *Lamium purpureum* possess hepatoprotective potential. The extracts of these species containing polyphenolic compounds, also have anti-inflammatory and antioxidant

properties, of free radicals neutralizing, as well as antiproliferative properties (Matkowski and Piotrowska, 2006; Yalcin and Kaya, 2006).

Increases of TGO and TGP transaminases serum levels in most experimental groups without being accompanied by hepatic parenchymal morphostructural changes can be a result of the animals' exposure to stress. TGO and TGP elevated values does not automatically mean a liver disease, mild increases of TGO may occur in some forms of muscular dystrophy in dermatomyositis, trauma, various forms of stress etc. (Fischbach, 2009; Kumar et al., 2012).

In conclusion, liver histological analysis results showed that administration of *Lamium purpureum* and *Lamium album* extracts in condition of exposing the animals to the immobilization stress had no adverse effects on the liver structure.

Analysis of Biochemical results

As mentioned above, serum transaminases show significant changes in TGP case, whose value increased significantly compared to the control group in all four treated groups, respectively with 24.75% ($p<0.01$) in group S, 19.96% ($p<0.05$) in group LA, respectively with 64.48% ($p<0.001$) in group SLA intoxicated.

TGP level does not suffer major changes in values compared to controls, only in the SLA group it increases significantly, with 49.65% ($p <0.001$).

As it is known, the liver is an important organ for metabolism and detoxification. It contains considerable amounts of polyunsaturated fatty acids, which are likely to be destroyed by free radicals. TGO and TGP are mainly markers of liver function.

In our study TGO and TGP increased activity demonstrated the liver damage in rats exposed to immobilization stress. This could be due to the alteration of cell membrane permeability which allow these enzymes to leak from the cell with intact membrane, when any stress or other liver cell damage exist, enzymes escape in the blood, and so, the enzymatic activity of TGO and TGP increases. These observations are in consensus with those of Nayanatara et al. (2009).

On the other hand, elevated values of TGO and TGP do not necessarily mean the liver damage. One reason may be hypovolemic shock. TGO is normally found in tissues such as the liver, kidney, muscle, heart, brain. It is released if one of these organs is affected (http://www.reteauamedicala.ro/simptome/92-interpretare-analize/2257-transaminazele).

These considerations make us assume that elevated values of TGO in S, LA, SLA and SLP groups is due to stimulation of diuresis, dead nettle extract causing a large diuresis, which led to dehydration, which entailed a number of

changes including hematologic ones. In animals belonging to SLA and SLP group, the increased value of TGO versus the S and LA groups, might be explained by the fact that anakinetic stress state is added, animals being exposed to this.

Following the applied treatments, it is found that significant changes in value at glucose and total protein (TP) level do not occur.

Serum cholesterol levels increase significantly (+ 24.73%, p = at limit) in group S, slightly decreased in the group LA (-8.32%, p>0.5) and significantly lower in LP group (-41,60%, p<0,5) than the C group, and also significantly decrease (-55.47%, p<0.001) in SLA group and with -43,50%, (p<0,01) in SLP group. Triglyceride concentration is increased in the five experimental treated groups, with 123.95% (p = limit) in group S, 123.16% (p = limit) in group LA, 111.35% (p<0.05) in SLA group, 72,49% (p = limit) in LP group and with 100,08% (p<0,05) in SLP group.

Regarding these aspects, numerous experimental studies on animals have provided empirical support for defining a relationship between stress and lipid concentrations. Cholesterol level was significantly increased in S group, similar to that reported by other authors (*Jain et al., 2000*).

It was suggested that different forms of stress increase cholesterol level by disturbing the synthesis and excretion rate (Champe and Harvey, 1994.). Different authors have suggested that these changes are due to epinephrine effect on lipoprotein lipase, hormone-sensitive lipase and hepatic lipase (Lundberg et al., 1989; Muldoon et al., 1995). Muldoon et al. suggested that psychological stress determined the volume decreases, causing haemoconcentration that may be the secondary cause of the increase in cholesterol levels (Muldoon et al., 1995).

It is known that catecholamine activate lipolysis in adipose tissue and increase the flow of free fatty acids to the liver where there is increased synthesis and secretion of triglycerides. High triglyceride levels observed in this study could be due to catecholamine-induced by anakinetic stress.

Decreases of cholesterol concentration and increases of triglycerides level were obtained by other researchers following administration of *Lamium album* (Pashazadeh and Rezael, 2013). As it is know, white dead nettle is used as antihypertensive, antihyperlipidemic and antidiabetic plant. Therefore through its positive effect on lipid profile, dead nettle may improve metabolic syndrome (Ahangarpour et al., 2012). In LA, LP SLA and SLP groups increases of triglyceride levels may be due to diuretic and anti-inflammatory effect of *Lamium album* extract (http://www.sfatulmedicului.ro/Colesterolul-si-trigliceridele/trigliceridele_953) which entailed a decrease in plasma volume and implicitly the increase of the blood triglycerides concentration.

Conclusions

Following the administration of *Lamium album* and *Lamium purpureum* plant extracts as well as the animal treatment with those two extracts concomitantly with stress exposure did not reveal significant structural morphological changes of hepatic parenchyma compared with controls.

Elevated TGO values occurred probably due to alteration of membrane permeability, on one hand, and on the other hand to hypovolemia occurred due to diuresis stimulation by red dead nettle extract.

The obtained biochemical changes are related to the morphology of the studied organ.

Rezumat. Scopul studiului nostru s-a axat pe analiza potenţialului protector al extractului hidroalcoolic de *Vaccinium vitis idaea* L. asupra unor parametrii biochimici sanguini (nivelul glicemiei, colesterolului, creatininei şi ureei, precum şi activitatea enzimelor hepatice GOT şi GPT), la şobolanii albi Wistar intoxicaţi cu alcool etilic şi trataţi cu extract hidroalcoolic 1:1 de *Vaccinium vits idaea* L (merişor) (200 mg s.u./100 g greutate corp). Extractele au fost administrate timp de 15 zile la şobolani albi Wistar femele, în greutate de 150 ± 10 g, sau în acelaşi timp cu alcool 50^0 (6g/kg corp.). Am găsit că simpla administrare a extractului vegetal de merişor nu determină efecte toxice, în timp ce tratarea animalelor cu extract de merişor a avut efecte de protecţie, prevenire şi imunostimulare la aproape toate nivelele parametrilor luaţi în studiu, în condiţiile intoxicării cu alcool.

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INFECTIONS ASSOCIATED WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION - A SHORT REVIEW

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Abstract. Immunodeficiency viruses (HIV), which are causing immunodeficiency syndrome (AIDS), represent the result of viral transmission between species from non-human primates to humans. HIV is originating in rural Africa and took birth from a mutation of a relatively benign virus, hosted by primates. The aim of this work is to look over the affections which are due to HIV infection. Thus, these are classified in two categories: affections due to direct involvement of HIV and opportunistic ones, caused by immunosuppression, which comprise opportunistic infections and neoplasias. These affections are due to a series of microorganisms which cause different diseases.

Key words: HIV, infection, opportunistic infection, tumoral infection.

Introduction

The human immunodeficiency virus (HIV) has a worldwide impact. The infection leads to the acquired immunodeficiency syndrome (AIDS). The patient usually remains asymptomatic a long time span after the infection begins, while the body's immune system tries to control it.

The immune system being debilitated, opportunistic infections set in. Out of these infections, the most common are: tuberculosis, various candidiasis, multiple forms of lymphoma and *Herpes simplex* or zoster (Gail et al., 2015). Regarding tuberculosis, HIV is the greatest risk factor for reactivation of previously dormant infections and it accounts for about 26% of HIV/AIDS-related deaths, being a leading cause of death for HIV-positive pacients (Pawlowski et al., 2012).

In HIV infection take place a series of congruent biological processes, which can be framed in three essential aspects:

- viral replication in the cells of the host;

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- replication of cell extensive infection on the immune defense system;
- consequences of the collapse of the immune defense capacity.

Due to these biological processes affections resulted from HIV infection.

Infections are common causes of morbidity and mortality in autoimmune diseases, and opportunistic infections are the tip of this problem, emerging as one main causes of morbidity in these patients (Faria et al., 2015).

Out of affections due to HIV implication, some appear during retroviral acute infection (meningitis, encephalitis, polyradiculoneuritis), others in the final stage of the disease, their incidence and severity being influenced by the immunosuppression degree (HIV encephalopathy, HIV myelitis, medullary suppression, HIV cardiomiopathy, HIV nephropathy, HIV arthropathy).

Opportunistic affections, represented by opportunistic infections and neoplasia, characterize the final stage (AIDS) of HIV infection, in most cases causing the death of a HIV seropositive person. In the category of opportunistic infections associated to HIV infection were included both proper opportunistic infections, which are conditioned by the state of pronounced immunosuppression, not affecting immunocompetent persons, (comprising infections with *Pneumocystis jirovecii*, atypical mycobacteria, JC virus, *Cryptococcus neoformans, Cryptosporidium parvum* etc.), and infections which appear also to immunocompetents, (tuberculosis, toxoplasmosis, herpes viruses infections, diverse bacterial infections), but to HIV seropositives they develop different, usually much more seriously, persistent, recidivating (Zanc, 2011; Zanoni and Gandhi, 2014).

Microorganisms such as *Mycobacteria* are causing diseases that are considered definitely opportunistic (e.g. disseminated tuberculosis) but also ones that are not considered opportunistic (e.g. pulmonary and pleural tuberculosis). Due to the fible immune system opportunistic infection need a deficient host (Faria et al., 2015).

1. Opportunistic infections

Opportunistic infections are caused by non-pathogenic microorganisms (bacterial, viral, fungal, or even protozoan) that can become pathogenic when the immune system is feeble, due to a prior cause as cancer, diabetes, HIV infection, and other immunodeficiencies.

1.1. Protozoa infections

Toxoplasmosis is in most cases the manifestation of the reactivation of a latent infection, when the CD4 number falls below 200/mm^3. Toxoplasmic encephalitis is the most frequent cause of focal infection of the central nervous system at a HIV infection patient, manifesting cerebral abscesses usually multiple, which condition a wide variety of neurological signs, within a clinical situation relative unspecific, insidiously developed. Other manifestations of

toxoplasmosis: lingering interstitial pneumonia, pleuresia, retinochoroiditis, myocarditis, panhypopituitarism, diabetes insipidus, inappropriate antidiuretic hormone secretion, orchitis, ascites, diarrhea, acute liver failure, acute pancreatitis, hemorrhagic, cystitis, erythema eruption. The treatment of cerebral toxoplasmosis lasts 3-6 weeks, recommended is the association of pyrimethamine with sulfadiazine and folic acid, followed by secondary prophylaxis with the same medication. The primary prophylaxis with *trimethoprim/sulfamethoxazole* (TMP-SMX) of toxoplasmosis is carryed out in the presence of positive IgG-anti-toxoplasma and a number of CD4 below 200/mm^3 (Zanc, 2011).

Cryptosporidiosis caused by *Cryptosporidium parvum,* usually cause diarrhea, cholecystitis, pancreatitis, pneumonia. Specific treatment (paramomycin) is much less efficient than immune reconstruction by HAART (Zanc, 2011).

Isosporidiosis, caused by *Isospora belli,* is an important cause of obstinate diarrhea, severe, at AIDS patients from Africa, Central and South America. It is treated with *TMP-SMX*, requiring secondary prophylaxis (Zanc, 2011).

Cyclosporiasis is a cause of severe prolonged diarrhea, necessitating treatment with *TMP-SMX*.

Microsporidiosis usually causes chronic diarrhea along with weight loss, and sometimes keratoconjunctivitis, hepatitis, sinusitis, cholangitis, renal or genital infections, or disseminated disease (Zanc, 2011).

1.2. Fungal infections

Invasive fungal infections (IFI) represent a main cause of global HIV mortality. Despite launching of combined antiretroviral therapy, there are still recorded up to 1 million deaths per year due to IFI. IFI are responsible for 50% of HIV related deaths. An historic failure of the focalization of efforts on IFI, which kills such a huge number of HIV infected patients, has created fundamental deficiencies in the management of advanced HIV infections (Armstrong et al., 2014).

Infection with *Pneumocystis jirovecii* (*carinii*) is frequent at HIV seropositive patients with severe immunosuppression (CD_4 <200/mm^3), usually manifesting through interstitial pneumonia which often evolves with severe respiratory insufficiency. Seldom it can cause: external ear polyposis, mastoiditis, choroiditis, necrotizing vasculitis similar to Buerger disease, medullary hypoplasia, hilar or mediastinal lymphadenopathy, pancytopenia, thyroiditis, hepatitis, intestinal obstruction etc. The election treatment consists in trimethoprim sulfamethoxazole (TMP-SMX) for 21 days. The primary prophylaxis of the infection, with TMP-SMX, is applied to all patients with CD_4 number below 200/mm^3 (Zanc, 2011).

It was proven that more than 90% of *Pneumocystis* pneumonia (PCP) in adults appears along with the chronic HIV infection with CD4 below 200 cells/ml. Even if the primary infections can cause CD4 profound transitory limphocytopenia, PCP and other opportunistic infections, it is seldom reported in primary HIV infections. These studies have shown the primary HIV infection can lead to severe immunosuppression, resulting also opportunistic infections. It is desirable to detect HIV infections in the primary stage, especially those coupled with PCP, which is usually detected in the final stages at the HIV infected patients (Ungprasert et al., 2013).

Candidosis is the most frequent fungal infection of HIV seropositive patients, its manifestations depending on the degree of immunosuppression. In the initial stages of HIV infection it causes local effects, on mucous membranes (stomatitis, pharyngitis, vaginitis), and in the advanced stages ($CD_4 < 100/mm^3$) esophagitis, respiratory diseases (tracheitis, bronchitis, pneumonia), fungemia. The antifungal treatment depends on the location of the infection and the immunosuppression degree, in severe cases necessitating carrying out an antifungal susceptibility test. The most efficient prophylaxis is immune reconstruction (Zanc, 2011).

Cryptococcosis (caused by *Cryptococcus neoformans*) is the major cause of meningitis at AIDS patients, seldom causing focal brain injury. The pulmonary action manifests through alveolar or interstitial pneumonia, pleuritis or hilar and mediastinal adenopathy. The election treatment for cryptococcocal meningitis consists in administration of *amphotericin* B and *flucytosine* for 14 days, followed by *fluconazole* treatment for 8 weeks, than secondary prophylaxis with *fluconazole*. Primary prophylaxis is not recommended (Zanc, 2011).

Histoplasmosis (produced by *Histoplasma capsulatum*) causes a clinical picture depending on the immunosuppression degree. The localized form can manifest through: meningitis, encephalitis, diarrhea, abdominal pains, intestinal obstruction or perforation, peritonitis, varied cutaneous eruptions, pericarditis, pancreatitis, retinitis, prostatitis. Disseminated histoplasmosis (usually $CD_4 <200/mm^3$) evolves with weight loss, hepatomegaly, adenopathy, meningitis or focal brain injury, oral and intestinal ulcerations, pneumonia. The treatment, with B *amphotercin* or *itraconazole*, is followed by secondary prophylaxis with *itraconazole* (Zanc, 2011).

Coccidioidomicosis (caused by *Coccidioides immitis*) affects the patients with severe immunosuppression ($CD_4 <50/mm^3$), usually manifesting by prolonged pneumonia, meningitis, more rare by cutaneous, ganglion, hepatic, osteoarticular affectation. The antifungal treatment depends on the severity and localization of the infection, being followed by secondary prophylaxis with *fluconazole* and *itraconazole* (Zanc, 2011).

1.3. Viral infections

Infections with cytomegalovirus (*CMV*) usually represent the reactivation of a latent infection, in a state of severe immunosuppression (CD_4 <50/mm^3). CMV retinitis causes cecity, in absence of pain or photophobia, by retinal necrosis followed by cicatrisation and sometimes retinal detachment. Frequent CMC is a cause for esophagitis, colitis, pancreatitis, interstitial pneumonia, encephalitis, myelitis and more rare hepatitis. The election treatment is represented by ganciclovir, followed by secondary prophylaxis with oral *ganciclovir* (Zanc, 2011).

Infection with Herpes simplex viruses can result in recurrent mucocutaneous injuries, esophagitis, recurrent panaritium, necrotizing retinitis, encephalitis, less often hepatitis, pneumonia, meningitis or myelitis. The election treatment is acyclovir. The acyclovir resistant strains are treated with *cidofovir* or *foscarnet* (Zanc, 2011).

Infection with Epstein-Barr virus (VEB) can manifest as infectious mononucleosis, oral hairy leukoplakia, or, in severe immunosuppression (CD_4 <200/mm^3), can cause non-Hodgkin systemic lymphomas or primary lymphoma of the central nervous system. *In vitro*, VEB is sensitive to *foscarnet, ganciclovir* and *penciclovir,* but the infection usually does not demand specific treatment. The caused by VEB demand oncological treatment, not antiviral (Zanc, 2011).

Infection with varicella-zoster virus (VVZ) manifests according to the degree of immunosuppression as varicella (with different degrees of severity), zoster herpes, atypical cutaneous injuries, necrotizing retinitis, as well as affecting the central nervous system (by meningitis, encephalitis, myelitis, polyradiculoneuritis, cerebral vasculitis) either during a varicella or zoster herpes development or at weeks or month after a zoster herpes episode, or even in the absence of a cutaneous manifestation VVZ chacteristic. The election treatment is represented by *acyclovir*, and in the case of strains resistant to it, by *foscarnet* or *cidofovir* (Zanc, 2011).

1.4. Bacterial infections

Bacterial infections represent a major death cause at AIDS. The most frequent manifestations of bacterial infections are represented by gastroenteritis, respiratory tract infections and sepsis. Generally, the HIV infected patient has a higher predisposition to all bacterial infections, specially to those encapsulated, presenting, as well, a higher risk for the evolution of a bacterial infection to sepsis. The bacteria which cause the most severe infections to the HIV infected patient are: pneumococcus, staphylococcus, *Salmonella* sp., *Campylobacter* sp., and *Pseudomonas* sp.

Tuberculosis, together with cryptococcal infections, are opportunistic infections that most commonly develop at patients living with human

immunodeficiency virus infection around the world (Zanoni and Gandhi, 2014). Tuberculosis is an often cause of death to HIV infected patients. To these patients, it can represent a primary infection or a reactivation of a latent infection. Tuberculosis can start in any stage of HIV infection, usually at a lesser degree of immunodeficiency than in the case of other opportunistic infections. On average, the number of T-CD_4 lymphocytes being in the range 150-350/mm^3 at the moment of tuberculosis diagnostic. The tuberculosis risk is considered to be 25-30 higher at HIV infections than in the general population, justifying the indication of HIV testing at any patient with newly diagnosed tuberculosis and, as well, the periodic evaluation of HIV patients for detecting a tuberculosis infection. The clinical manifestations are generally similar with those of HIV seronegative patients, but the incidence of extrapulmonary tuberculosis localization is much higher, in parallel with the degree of immunosuppression on which it evolves, affecting in medium 30-60% of cases (Trinha et al., 2015). The tuberculostatic treatment is generally similar with the one at HIV seronegative patients, pointing out that *rifampicin* can not be administrated at a patient treated with a protease inhibitor. Thus, if it is not possible to modify the scheme of antiretroviral therapy so it does not contain a protease inhibitor, *rifampicin* is replaced with *rifabutin*, which does not interact with protease inhibitors. The time lapse for tuberculosis treatment is generally 6-9 months, sometimes longer, according to the chosen therapeutic scheme and evolution (Zanc, 2011).

If during 2012, in Colombia, the tuberculosis diagnosis was confirmed in 6.4% of cases (Arenas et al., 2012), in 2015 the coinfection HIV with tuberculosis was 9.1% and HIV with histoplasmosis was 6.2% (Agudelo-Gonzalez et al., 2015).

Infections with atypical mycobacteria affect the patient with HIV infection in the final stage, the number of CD_4 lymphocytes being generally under 100/mm^3, especially if another opportunistic infection is concomitant present. Unlike *Mycobacterium tuberculosis*, atypical mycobacteria are opportunistic germs, widely outspread in nature. The species most involved in HIV seropositive infections are: *Mycobacterium avium* complex, (association between *Mycobacterium avium* and *Mycobacterium intercellulare*), *Mycobacterium kansasii, Mycobacterium xenopi, Mycobacterium chelonae* etc. Atypical mycobacteria usually diffuse respiratory or digestive way and cause a clinical picture dependent on the degree of immunosuppression of the host. The patients with the number of CD_4 lymphocytes above 50/mm^3 usually present a localized infection, manifested by lymphadenitis, mostly cervical or subclavicular, pneumonia, diarrhea, meningitis, osteomyelitis, pericarditis or cutaneous abscesses. With some variations depending on species, atypical mycobacteria are sensitive to *clarithromycin*, *azithromycin*, *rifampicin*, *isoniazid*, *ethanbutol, fluoroquinolone*, *amikacine*. The treatment comprises at least two antimicrobial agents, at infection with *Mycobacterium avium* complex

are preferred *clarithromycin* and *etambutol*, to which a third antibiotic is added if the CD_4 number is below $50/mm^3$, either *rifabutin* or *amikacine*, *ciprofloxacin* or *levofloxacin* (Zanc, 2011).

Bacillary angiomatosis, determined by *Bartonella* sp., frequently affects the HIV infected patients, which can acquire infection even without cat scratches or bites, probably by direct contact, the germs being spread also in soil. Clinical manifestations include: diverse cutaneous injuries (most often red-violaceous papilla), osteolytic injuries, erythematous nodules at mucous level, pancytopenia, abscess, hemorrhagic cysts or necrosis at hepatic or splenic level, meningitis, bacteremia and endocarditis. The treatment is carried out with erythromycin or doxycycline, azithromycin, lasting at least 3 months (Zanc, 2011).

2. Tumoral affections

At HIV infected patient the risk of developing, both neoplasias and premalignant affections, is considerably grown, proportionally with the degree of immunosuppression.

Kaposi sarcoma is a multicentric neoplasia made of multiple vascular nodules which can affect the tegument, the mucous membranes. Although is admitted that the most common form is the cutaneous one, the disease can develop anywhere in the organism, the most frequent executor situs being lymphatic ganglions, lungs and gastrointestinal tract, the presence of visceral affection being estimated at 30-80% (Schoefer et al., 2007). Sometimes, sarcomatosis visceral injuries precede the cutaneous ones (Cupşa and Chiurtu, 2010). Infection with human herpes virus 8 (HHV-8), which affects almost exclusively the male homosexuals, is implicated in Kaposi sarcoma pathogenesis, HHV-8 being considered cofactor of neoplasia triggering. Kaposi sarcoma appears in any stage of HIV infection and is rarely invasive, the patients usually dying by other opportunistic infections. The clinical picture is extremely varied, consisting in the presence of red nodules at the level of tegument (preferentially in the zones exposed to sun or prior traumatized), mucous membranes, lymphatic ganglions and, practically, any organ, frequently complicating with hemorrhage, obstructions. The diagnostic is histo-pathological and the antitumoral therapy consists in chemotherapy and, at pulmonary affections, radiotherapy, a favorable effect having immune reconstruction under HAART. The actual treatment does not cause healing of Kaposi sarcoma, the aim of therapy being to reduce symptomatology and disease progression (Zanc, 2011).

Non-Hodgkin systemic lymphoma has an incidence 120 times bigger at untreated HIV patients than in the general population, happening against a severe immunosuppression. These lymphomas are heterogeneous entities with variable replication rate, 70% being lymphoma with B cells, and 30% with T

cells. Epstein-Barr virus is involved in oncogenesis being present at about 40% of cases. The symptomatology is very diverse, any organ can be affected, 80% of cases having extraganglionic location: central nervous system, (30% of cases), gastrointestinal tract (25%), bone marrow (20%), liver, lungs etc. The diagnostic is histopathologic, and the treatment consists in chemotherapy, according to the number of CD_4 lymphocytes (Zanc, 2011).

Hodgkin lymphoma (HL) continues to cause substantial morbidity and mortality, even though it is a curable disease (Hutchings et al., 2015).

Primary lymphoma of central nervous system begins in conditions of severe immunosuppression, the implication of Epstain-Barr virus being also proved by its presence in all the cases. Cerebral affectation is predominant, causing a neurological picture dependent on location: initially confusion, lethargy and memory damage, than can develop hemiparesis, aphasia, convulsions, cranial nerves paresis and cephalalgia. Differential diagnostic is established with cerebral toxoplasmosis, sometimes being extremely difficult. Imaging and identifying Epstein-Barr virus DNA in LCR are essential for diagnostic. By histo-pathological examination is concluded the final diagnostic but it requires cerebral biopsy, risky invasive procedure, especially in some locations of the focal formation. The treatment is oncological, radiotherapy being applied, sometimes associated with chemotherapy, the affection being associated with medium survival of 3 months (Thormar, 2013; Gendelman at al., 2011).

Intraepithelial dysplasia of cervix and anus, proved on Papanicolau smears, is associated with human *Papilloma* viruses, the evolution to an invasive neoplasm being possible.

Other tumors A multitude of neoplastic diseases have been described at HIV infected patients, but none has a higher incidence than in the general population. The evolution is more severe and the treatment more difficult at HIV patients (Zanc, 2011; Arbune and Georgescu, 2013).

Systemic lupus erythematosus (SLE) and HIV infection have many common clinical manifestations, including myalgia, arthrosis, cutaneous eruptions and lymphadenopathy. It also affects organs as kidneys, heart, lungs and central nervous system. They also have common lab results as anemia, leukopenia, lymphopenia, thrombocytopenia and hypergammaglobulinemia. Autoantibodies, as antinuclear antibodies (ANA) and anticardiolipin antibodies, which usually appear at SLE patients, can also be present to HIV infected patients. The treatment for SLE at persons treated with immunosuppressive drugs can lead to apparition of not prior diagnosed HIV or the progression of viral replication at HIV infected patients. Using antiretroviral therapy at HIV patients can be associated with reactivation or new start of SLE as result of re-establishment of immune function (Mody et al., 2014).

HIV infected patients present the risk to develop cardiovascular diseases three times higher than people not infected with this virus. HIV infection is also

associated with a high risk for precancerous injuries and for cervical cancer at women (Costiniuk et al., 2013; Hanischa et al., 2013).

Rezumat. Virusurile imunodeficienţei umane (HIV), care sunt agenţii cauzali ai SIDA, sunt rezultatul transmiterilor virale între specii de la primate non-umane la om. HIV este originar din Africa rurală şi a luat naştere în urma apariţiei unei mutaţii dintr-un virus relativ benign, având drept gazdă maimuţele. Scopul acestei lucrări este acela de a trece în revista afecţiunile datorate infecţiei cu HIV. Astfel, acestea se clasifică în două categorii: afecţiuni datorate implicării directe a HIV şi afecţiunile oportuniste, datorate imunodepresiei, care cuprind infecţiile oportuniste şi neoplaziile. Aceste afecţiuni sunt cauzate de o serie de microorganisme care determină diferite boli.

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DETECTION METHODS FOR INDICATOR MICROORGANISMS IN DRINKING WATER – A REVIEW

Anca FLOREA*, Rahela CARPA*

Abstract. Although new, fast and efficient methods for detecting microorganisms in water were implemented and perfectioned, we face the same problems the researchers in 19th century faced: "which is the most efficient method to analyze water?", "which are the most suitable indicators?", "which is the direct relation between indicators and the pathogens present in water?", "how often water monitoring should be carried out?" etc.

Water is not important only for the daily consumption, but also in the food industry, where in used in different technological processes as raw material, etc. It must enframe into the potability parameters in order to insure the appropriate quality of the food stuffs and the safety of consumers. The control and monitoring of water quality aims keeping water within adequate potability parameters and implicitly avoiding the occurrence of new epidemics which endanger people.

This paper describes different analysis methods for detecting and counting the indicator microorganisms used for water potabilization.

Key words: MTM, membrane filtration, drinking water quality, indicator microorganism.

Introduction

Microbiological monitoring of water quality is performed using lab analyses – quantitative and qualitative.

Classical methods, standardized for identification and counting of microbiological indicators, are: the multiple tubes method and the membrane filtration method. Though prevalent these present limitations as: long duration, lack of specificity and the presence of bacterial antagonism (Rompré et al., 2002).

Due to encountered limitations, modern methods, enzymatic and molecular, are searched for. Though rapid and with high specificity (Kearns et al., 2008), usage costs are high.

Present-day are also the efforts to identify and direct detection of pathogens present in tap water.

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These new methods are tested out of the need to avoid eventual false-positive results at classic methods and to reduce the period of exposure to potential pathogens, prolonged due to the long lasting traditional methods. The fact that pathogens can be extremely infectious even in low concentrations (Leskinen et al., 2012) is also important. The numerous disadvantages which hinder the performance of the procedures and diminish the detection expectance are also taken into consideration.

The indicator organisms currently used are needed to determine if water is drinkable and microbiologically safe, if the treating methods for water are efficient, disinfection is adequate or the water sources are within parameters.

1. Multiple tubes method (MTM)

The quantitative analysis, based on the capacity of coliform bacteria and *Escherichia coli* to produce lactic acid and gas (CO_2), presents a 100 years tradition in monitoring the quality of drinkable water (Ashbolt et al., 2001).

The method implies inoculation of decimal dilutions of the water sample in nutritive culture media, 3 to 5 for each dilution (the higher the number of inoculated media, the bigger the preciseness) and observing the capacity of possible microorganisms to ferment lactose for 48 hours at 35^0C. The formation of gas from reaction is observed by introducing a Durnham tube before inoculation (Pepper and Gerba, 2005; Carpa et al., 2014).

Microorganisms as *Clostridium perfringens*, which is Gram positive and does not belong to the Coliform Group, ferment lactose. It is thus necessary the subsequent use of selective media (ex.: BBLVP medium, lactose bile, brilliant green), which to inhibit the development of Gram positive bacteria (Pepper and Gerba, 2005).

All these steps are carried out in two steps – presumptive and of confirmation – which take a long period due to 48 hours incubation periods within each step. Another limitation is represented by inhibitory effects of certain media and the large number of microorganisms which can exhibit false positive results.

Assessing the number of organisms, within this method, is performed through estimations and biostatistic analyses. The colonies are not properly numbered, but the result is assessed follow-up counting of positive samples by the technique of the most probable number (NPM). It must be pointed out that the numeric results obtained are not precise and at assessing the colonies on solid media these are much more reduced in number (Okafor, 2011).

Even though it presents numerous disadvantages, the method of multiple tubes is still popular within microbiological investigations because it allows the use of a large range of media. Furthermore, it presents a wide range of applications being successfully used also in counting *Staphylococcus aureus* present in foods (Bennett and Lancette, 1998).

2. Membrane filtration method

Membrane filtration method is a quantitative analysis which is alternative to the multiple tubes method.

It implies the filtration of a water quantity through a filtering membrane with 0.45 μm pores which hinder target microorganisms. This membrane is placed on a cone connected to the vacuum pump (Fig. 1). The filtered water volume depends on the probable density of microorganisms in water sample (Okafor, 2011). The membrane is then placed on a selective solid medium and incubated at specific temperatures and time periods.

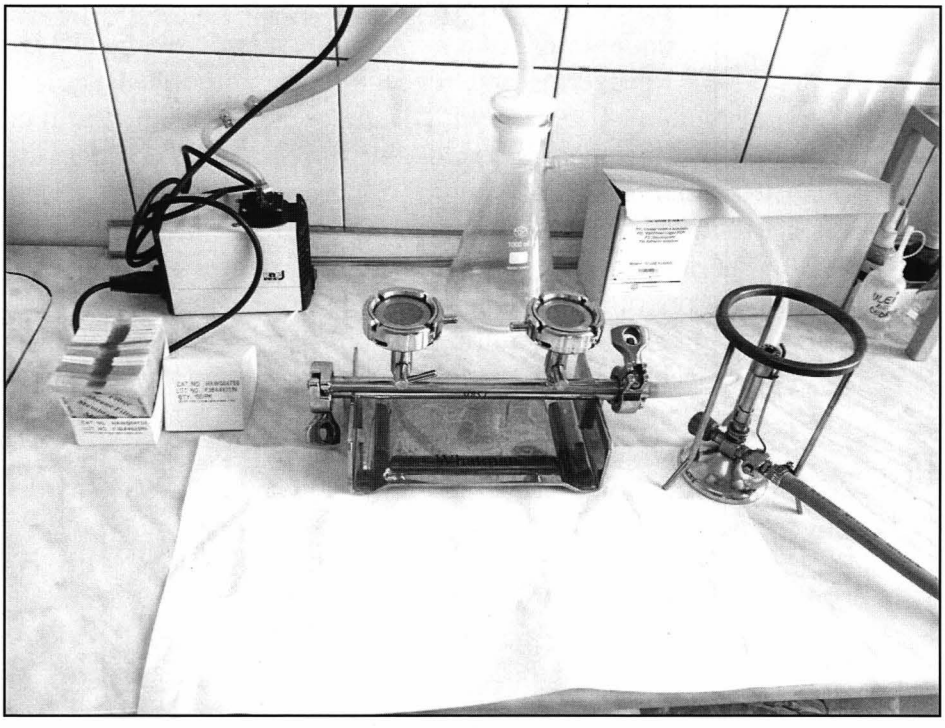


Fig. 1. The membrane filtration method – equipment

The effectiveness of this water microbiological analysis depends on the culture medium used, which allows the identification of probability indicators (Pepper and Gerba, 2005).

3. Other methods

The new rapid procedures tested require a big number of organisms without preliminary development on culture medium, which would increase the analysis time. Thus, there is the possibility to concentrate microorganisms out of large water volumes in order to grow the detection probability and to obtain the needed microorganisms quantity. Furthermore, the concentration of certain

microorganisms reduces the time lapse between sampling and detection, reducing the exposure to potential pathogens (Leskinen and Lim, 2008).

Ultrafiltering is the lab procedure whereby is performed the concentration of both indicator and pathogenic microorganisms. This procedure is based on the same principle as normal filtering: the molecules smaller then pores pass through membrane outside the filtering system, while larger items are retained and concentrated (Morales-Morales et al., 2003).

There are two particular types of ultrafiltration:

- CFF (cross-flow ultrafiltration) is a continuous process in which big water quantities are recirculated at the surface of the membrane until obtaining a minimum required volume (Kearns et al., 2008). Recovering and concentrating microorganisms is done by recirculating a buffer. An advantage of this method in relation to DEUF is that there is no clogging of pores due to recirculating.
- DEUF (dead-end ultrafiltration) is a process in which water passes once through the filtering membrane without being recirculated. Microorganisms caught in the membrane pores are recovered by washing on runback with water or buffer (Leskinen and Lim, 2008). Even though during experiments decreases of filtering flow happened due to settling by organic or inorganic materials (Kearns et al., 2008) DEUF is more efficient than CFF due to the rapidity of performance. The efficiency of recovering and concentrating microorganisms in DEUF case is 57-94% and it has generally the highest value for water with low turbidity, respective the lowest for water with medium turbidity (Smith and Hill, 2009).
- In order to reduce the time lapse between recovering and processing a DEUF model for field analysis: PMACS (Portable Multi-use Automated Concentration System) (Leskinen et al., 2012).

After collecting the concentrate, it is subjected to specific analyses for identification of indicator and pathogenic microorganisms. The larger the water volume subjected to ultrafiltering, the higher the precision of the results regarding water quality (Leskinen et al., 2012, Leskinen and Lim, 2008).

3.1. Enzymatic methods are used for quicker identification of microbiologic indicators. Because of their reduced specificity those were combined with classic methods (Ashbolt et al., 2001).

The main metabolic reactions followed are due to the enzymes: β-D-glucuronidase, which catalyzes β-D- glucopyranosiduronic acid hydrolysis, and is specific to *Escherichia coli*, and β-D galactosidase, which catalyzes lactose the split of lactose into glucose and galactose, being specific to coliform bacteria (Rompré et al., 2002).

The presence or activity of these enzymes in highlighted using fluorogenic and cromogenic substrates. These substrates are used as vital nutrient only by the target organism (Rompré et al., 2002). After modifying the fluorogenic and cromogenic substances by corresponding enzymes specific colour of fluorescence will be emitted, which will indicate the presence of target organisms.

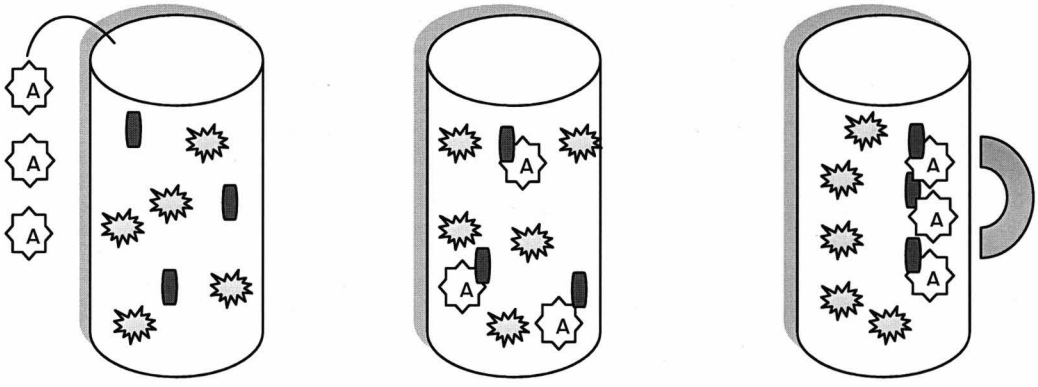
An example is the *qualitative test present/absent* in which two combined substrates are used: ONPG (*o*-nitrophenyl-β-D-galactopyranoside) for marking out coliform bacteria, and MUglu (4-metilumbelli-feril-β-D-glucuronide) for marking out

Escherichia coli (DeZuane, 1997). The method consists in adding the sample over the combined substrates and incubation at 35 °C. The yellow color indicates the hydrolysis of ONPG substrate and thus the presence of coliform bacteria. The tubes considered positive for coliform bacteria are then exposed to UV light of 366 nm. The release of white-blue fluorescence caused by 4-methylumbelliferone compound (MU) demonstrates the presence of *Escherichia coli*.

3.2. Immunological methods based on antigen-antibody relation present higher specificity and affinity, but are more laborious and costly. Nevertheless, numerous studies are performed in order to find an optimal method of identification for indicator organisms. The most examined methods are immunofluorescence assay and immunomagnetic separation (IMS).

IFA allows identification and detection of a single cell out of the sample by bonding an antibody with fluorochrome at antigen or by bonding an antibody to antigen and further attacking of initial antibody with one conjugated with fluorochrome (Rompré et al., 2002).

IMS involves introducing spheres covered with monoclonal antibodies in the sample, on which antigens and implicitly cells will bind (Fig. 2). After magnetical separation, target organisms can be grown and subjected to further counting and identification analyses. Using polyclonal antibodies (selected from more cell series) is avoided because they can react with related microorganisms (Bushon et al., 2009). Due to binding mechanism based on hydrophobic adsorption which can by chemically and physically destabilized more stable covalent binding mechanisms were developed (Lee et al., 2010).



Add antibody-coated beads to water sample.	Target bacteria (antigens) captured by the beads.	Complex attracted by a magnet.

Fig. 2. Immunomagnetic separation

IMS was coupled with numerous other detection methods for growing the efficiency, but the most studied was IMS/ATP-bioluminescence. At this method, after magnetic separation and isolation, the cells are broken and ATP is quantified. By adding luciferase, which in the presence of luciferine and oxygen catalyzes an ATP consuming reaction, light will be emitted, with intensity correlated to the concentration of the cells present in sample.

The technique is one of the most rapid modern methods, needs an hour of processing time (Bushon et al., 2009), allows numbering of viable cells , and the costs are much less than the ones at other molecular methods (Noble and Weisberg, 2005). It was used with success in detecting *Escherichia coli* O157:H7, *Giardia, Cryptosporidium parvum* (Lee et al., 2010).

3.3. Molecular methods. Molecular methods are based on detecting several sequences of nucleic acids and are used only experimentally.

These methods are based on PCR technique, which was adapted according to requirements, for an increased sensibility and specificity, for a reduced effectuation time and for simultaneous identification of more target microorganisms, even of new strains (Shi et al., 2010).

PCR technique implies in vitro amplification of a specific sequence from the genome of pathogenic microorganisms and of the indicator. This exponential amplification increases the detection possibility of the target sequences out of the samples with reduced number of microorganisms (Silva and Domingues, 2015).

The method involves more steps: concentrating from the sample the organisms of interest, extracting the nucleic acids from target organisms, purifying in order to eliminate the inhibitors, amplifying the target sequence and detecting or quantifying the amplified segments (Girones et al., 2010).

The main role in identifying the target organisms is taken by the oligonucleotide primers based on genes: *lacZ*, codifying β-D-galactosidase enzyme specific to coliform bacteria, and *uidA*, codifying β-D-glucuronidase enzyme specific to *Escherichia coli* (Cabral, 2010). The traditional methods also take into consideration these enzymes, but PCR technique detects several *Escherichia coli* strains which have the *uidA* gene, but do not have β-D-glucuronidase activity (Silva and Domingues, 2015).

Recently became possible to count the target sequences present in the sample through qPCR (Girones et al., 2010; Silva and Domingues, 2015, Shi et al., 2010). So the technique does not have only quantitative characteristics, but also qualitative.

One of the major advantages of molecular methods is the identification of VBNC (viable but non-culturable) organisms. These types of organisms are viable, but due to some metabolic changes given by certain factors (e.g.: toxic agents, lack of nutrients, instable temperatures) does not form colonies on culture media (Keer and Birch, 2003).

Even though it presents substantial advantages compared to other methods, the technique has its limitations: the small volume (microliters) of sample utilized, the existence of inhibiting substances which can diminish the amplification efficiency, the physiological status of organisms.

It was noticed that DNA is stable and persists significant amounts of time after the death of the cells (Heijnen and Medema, 2009). So it was tried to adopt ARN_m as viability marker because it has a shorter lifespan than DNA (Keer and Birch, 2003). RT-PCR (reverse transcriptase PCR), qRT-PCR and NASBA (nucleic acid based sequence amplification) represent present techniques for detecting some ARNm target sequences in the analyzed sample (Heijnen and Medema, 2009; Keer and Birch, 2003; Silva and Domingues, 2015).

Most of the tests mentioned above are complex, require a wide range of devices and modern equipment, qualified staff and funds which are not available in developing countries, rural areas, settlements with low resources of emergency situations. The need to adopt faster tests, with low costs, which to reduce the time of exposure to the action of a potential pathogen lead to the development of tests which are simple, certain and portable, not necessitating handling in a lab.

Thus 44 tests have been identified, out of which 18 qualitative are based on present/absent test and 26 quantitative are based on counting the concentration of the present indicators (Bain et al., 2012).

The most recent test evaluated is "**the compartment bag test**", which implies the qualitative and quantitative analyses of a water sample, with *Escherichia coli* colonies identification by green-bluish colouring of the different volumes (1, 3, 10, 30, 56 ml) compartments in which the sample was

introduced together with the culture medium (Fig. 3). The concentration of present microorganisms is assessed in the test by the most probable bacteria number technique (MPN) (Stauber et al., 2014).

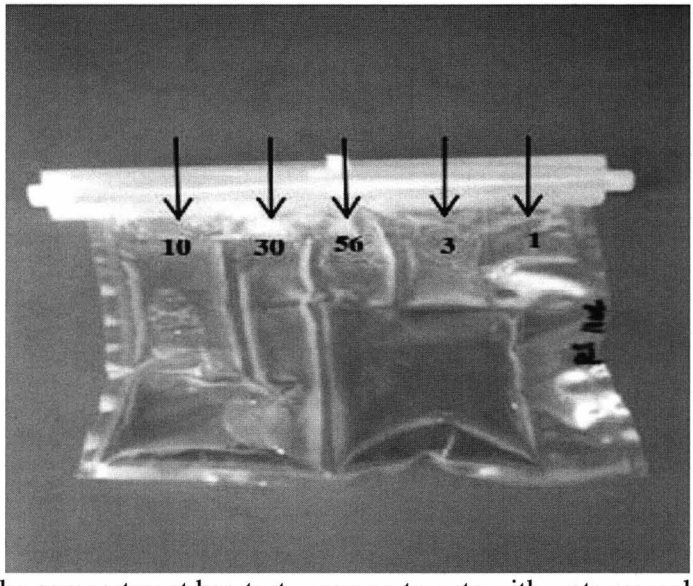


Fig. 3. The compartment bag test – compartments with water samples before incubation (Stauber et al., 2014)

The trials have proven that the test presents high stability and specificity, needs incubation at 35 °C (Stauber et al., 2014), is accomplished in a relative short time span of 18-24 h, is portable and the costs are low. "The compartment bag test" is thus suitable to be implemented as standard test in emergency cases or other cases of low resources.

Conclusions

In microbiology, concerning drinking water, several methods of detecting indicator microorganisms are used. The most frequently employed are:

- Multiple tubes method (MTM) – the classic method for determining indicator microorganisms;
- Membrane filtration method - which is a quantitative analysis alternative to the multiple tubes method;
- Enzymatic methods - the new rapid procedures tested require a big number of organisms without preliminary development on culture medium, which would increase the analysis time;
- Immunological methods - the technique is one of the most rapid modern methods, needs an hour of processing time, allows numbering of viable cells, and the costs are much less than the ones at other molecular methods;

- Molecular methods are based on detecting several sequences of nucleic acids and are used only experimentally.

Rezumat. Deşi s-au perfecţionat şi adoptat metode noi, rapide şi eficiente în vederea detectării microorganismelor din apă, ne confruntăm cu aceleaşi probleme cu care s-au confruntat şi cercetătorii din secolul al XIX-lea: „care este cea mai eficientă metodă de analiză a apei?", „care sunt indicatorii cei mai potriviţi?", „care este relaţia directă dintre indicatori şi patogenii prezenţi în apă?", „cât de des ar trebui să se realizeze monitorizarea apei?" etc.

Apa nu este importantă doar în consumul de zi cu zi, ci şi în industria alimentară unde este utilizată în diferite procese tehnologice ca materie primă, etc. Aceasta trebuie să se încadreze în parametrii de potabilitate pentru a asigura calitatea corespunzătoare a produselor alimentare şi pentru a asigura siguranţa consumatorului.

Controlul şi monitorizarea calităţii apei urmăreşte păstrarea apei în parametrii de potabilitate adecvaţi şi implicit evitarea apariţiei de noi epidemii care pun în pericol viaţa oamenilor.

Această lucrare descrie diferite metode de analiză pentru detectarea şi numărarea microorganismelor indicator utilizate în vederea potabilizării apei.

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SOME REMARKS CONCERNING THE ASIAN FISH SPECIES FROM ROMANIAN WATERS

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Abstract. In the present paper some aspects concerning the fish of Asiatic origin from the Romanian ichthyofauna are discussed. For each species we discussed its occurrence in our country, briefly the species biology, importance and possible impact on native fish species. The author did not took into consideration numerous species, subspecies, varieties of exotic fishes kept in aquariums in ornamental purposes, including the goldfish (*Carassius auratus auratus*) which is kept sometimes in small artificial pools.

Key words: alien species, impact, native species, occurrence, ichthyofauna.

Introduction

Out of the vertebrates, fresh water fish species weigh the most in the admissions (be they on purpose or not) of foreign species. There are around 40 species of fish entered into Europe, however, many more have been transferred from various European countries into some others (Holčik, 1991). The foreign species entered into Romania originate from North America, Asia (including the former Soviet Union) and Africa (Gavriloaie, 2007). The present work analyzes the foreign species of fresh water fish of Asian origin from Romanian waters. These species have entered the autochthonous ichthyo-fauna either naturally or introduced by human. In case of each and every species we discussed about the moment of its appearance in Romania and about how it has got there, as well as brief data on their biology, their importance and their impact upon the autochthonous species of fish. We have not taken into account the countless species, sub-species, varieties etc. of ornamental fish that are the subject-matter of the aquarium hobby, including here even the golden carp - *Carassius auratus auratus*, a species that is raised here and now in small pools in the open air.

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The Asian freshwater fish species entered into Romania

Thirteen species from this continent are now present in Romania, too. We present them below in the order of their appearance in the Romanian waters.

Cyprinus carpio (the carp) – penetrated in Europe thanks to the Romans, in a tight link with the spreading of Christianity. Alongside with the creation of the 12^{th}-13^{th} century monasteries, keeping the carp in ponds became a fundamental occupation, seeing that fish was an appreciated meal during fasting. In Romania the carp was the first species of fish, in its culture form, that was the subject-matter of acclimatization ever since 1300 (Manea, 1985) and it remained the basic species for the pisciculture practised in the hill and plateau areas until present days.

Carassius gibelio (the Prussian or silver carp) – originates from the Amur basin. It lives however in the largest part of the Siberia, of Eastern Europe and partially in Central Europe, in the Sâr-Daria and Amu-Daria basins, too. It is not autochthonous, but introduced in these regions, but the period when the introduction took place cannot be mentioned (Bănărescu, 1964). In Romania it was brought in 1912 from Bassarabia and introduced in the Fundeni lake (Pojoga, 1959). From here, in large waters, it passed in the Tătaru lake and then in Dâmboviţa, thus entering the Danube's area liable to being flooded. The strong flood of 1970 significantly contributed to the expansion of the silvery crucian in the Danube Delta (Oţel, 1977). At present this one is living in all categories of stagnant waters from the plains to the hill area, where it gradually replaced a related species, the *Carassius carassius* (the crucian carp) one; it does not quite thrive in the regions too invaded by the vegetation. It also lives in the plain rivers (in the region of the carp, occasionally in that of the dace, as well and in the one of the barbel, but in a pretty reduced number and solely in the areas with calm water. It is a frequent species in fish farms.

Coregonus lavaretus maraenoides and ***Coregonus albula ladogensis*** were imported in 1956 from the former Soviet Union under the form of embryoned eggs, which were distributed in the ponds of the Nucet and Tarcău piscicultural stations (Buşniţă et al., 1957) and in some lakes in Romania, where they are the used for sporting fishing, but we do not have more specific data on their present situation in Romania.

The **Chinese cyprinides** name includes the cyprinides species introduced in Romania in 1960 and 1962 from the Yang-Tze river in China and they were taken to the Nucet (Dâmboviţa) and Cefa (Bihor) piscicultural stations for the aquaculture. Several species were brought in, however only 7 were acclimatized. These ones are: *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Mylopharyngodon piceus*, *Parabramis pekinensis*, *Megalobrama terminalis* and a species incidentally brought in, namely *Pseudorasbora parva* (Manea, 1985). Out of these species, to the best of our knowledge, *Parabramis pekinensis* and *Megalobrama*

terminalis can no longer be found in Romania. It is likely that the *Hypophthalmichthys molitrix* and *Aristichthys nobilis* species reproduce naturally in the lower Danube (Bacalbaşa-Dobrovici, 2002) and *Ctenopharyngodon idella* (Giurcă, 1980), as well. *Mylopharyngodon piceus* is only preserved in fish farms, but in small amounts here, too.

Pseudorasbora parva (the topmouth gudgeon), is a small-sized (8.5-10.5 cm) bentophagous fish spread out in the entire Eastern Asia, from the Amur basin to the South of The People's Republic of China. The terra typica of this species is Nagasaki, Japan (Bănărescu, 1964). *Pseudorasbora parva* is a species with a high dispersion potential, which succeeded in spreading out in almost all the countries of Europe during the 45 years that passed from its admission into this continent. There were several centers in Europe, out of which the *Pseudorasbora parva* then spread out on almost the entire continent. The two major centers where Romania (from where the species naturally spread out in the whole Danube basin) and Albania (from where the species spread out in the Balkans, still naturally). In the countries of the former Yougoslavia the species penetrated from both centers; in Hungary, Slovakia and the Czech Republic the species penetrated both naturally, from Romania, and artificially, as it was brought straight from China, together with some other species of fish of an economical interest. In Poland and the Northern Bulgaria the species was seemingly brought from the Ukraine. The origin of the populations in Italy and France is unknown, but these populations probably come from the Danube basin. We assume that the species arrived in Denmark from Germany. We do not know how the species got on England's and Spain's territory, but it was most likely artificially introduced from a European country.

The considerable dispersion of this species in Romania was made by the introduction of the carp, of the silver carp etc. in various places. The species actively spread out through the hydrographical network, however the dispersion with the help of human played the most important role. In many cases the species was found for the first time in piscicultural basins and in their linking channels and only 1-2 years later in the adjacent rivers, too. The species also becomes abundent in some recreation lakes, where other species of a interest are introduced for amateur fishermen, such as the Kios lake in Cluj-Napoca or the Youth Lake in Bucharest. Even the amateur or sporting fishermen contributed to the enlargement of the realm of this species in Romania. The topmouth gudgeon is used as a bait for the predator fish and we sometimes noticed that the specimens that were still alive at the end of the fishing party were simply thrown into the water, but usually into another than the one from which they had been collected.

We have noticed that *Pseudorasbora parva* was highly abundent in the piscicultural enclosures and in the natural areas only in some lakes and small hill- and plain- rivers. We also found this species in the sub-mountain region, in

the Gurghiu river, but solely isolated specimens. In the larger rivers and even lakes it is present in small amounts. It feels comfortable in polluted areas, too, where few native species of fish survive (Gavriloaie & Chiş, 2006). Bănărescu (1993) states that the topmouth gudgeon has not yet caused the regression of any native species of fish.

Coregonus peled was introduced in 1980 by D. Matei within the Podu Iloaiei Piscicultural Research and Production Station under the form of eggs in the mobile embryo phase, imported from the former Soviet Union. *Coregonus peled* has only been bred in the ponds within the Podu Iloaiei P.R.P.S. (Matei & Manea, 1990) so far. We do not have any further data on this species.

Perccottus glenii (the Amur sleeper) – belongs to the Odontobutidae family, which is spread out in China, in the North-West of Korea, in the Amur basin and in Russia. In November 2005 a few juvenile specimens were collected from the Suceava river (Nalbant et. al., 2004), which may have come from the Ukraine. In 2005 a grown-up *Perccottus glenii* specimen was fished for in the Danube, at kilometer 929. This is the first sign of this species in the Romanian sector of the Danube (Popa et. al., 2006). Seeing the scarce information that we possess, we do not yet know how *Perccottus glenii* could influence the native ichthyofauna.

Conclusions

At present in Romania there are 13 fish species of Asian origin, out of which one (*Perccottus glenii*) penetrated naturally in virtue of the hydrographical network (and we do not have enough information so far about its ecological impact, because of the fact that it was spotted in the Romanian ichthyofauna a short time ago), and 12 species were deliberately or accidentally introduced by human.

As far as the foreign species of fish that have been artificially introduced are concerned, 6 of them naturalized themselves, which means that they are already reproducing in the natural waters as well, without human's help, locally or on larger areas (*Cyprinus carpio, Carassius gibelio, Ctenopharyngodon idella, Hypophthalmichthys molitrix, Aristichthys nobilis, Pseudorasbora parva*); one species only (*Mylopharyngodon piceus*) reproduces in piscicultural breeding enclosures, with the help of human; as for the *Coregonus* specis (*Coregonus lavaretus maraenoides, Coregonus albula ladogensis,* and *Coregonus peled*) we do not have enough data on their way of reproducing and establishemnt in Romanian waters. *Parabramis pekinensis* and *Megalobrama terminalis* are not present anymore in the country.

The real impact of the foreign fish species upon the native ichtyofauna is difficult to assess, as there are no research on the great majority of the eco-systems that existed before the penetration of the foreign species into them. For the time being we cannot surely allege that some native species have disappeared because of the foreign species of fish that have entered Romania. It

is true that the *Carassius carasius* species has disappeared from most of the lakes where the silver carp penetrated, but thorough research studies are needed in order to see whether the silver carp is the only cause of the crucian carp's regression. In some parts of Europe *Leucaspius delineatus* species regressed or even disappeared from the regions where *Pseudorasbora parva* penetrated, but in Romania this was not thoroughly studied.

For the future it is necessary to limit the introduction of new species of fish into the Romanian aquatic eco-systems as much as possible and, in case of the ones already entered, we ought to limit their spreading out in new habitats.

Rezumat. În cadrul acestei lucrări am analizat speciile străine de peşti dulcicoli de origine asiatică din apele româneşti. La fiecare specie în parte am discutat despre momentul apariţiei sale în România şi despre modul cum a ajuns aici, date sumare de biologie, importanţă şi impactul posibil asupra speciilor autohtone de peşti. Nu am luat în considerare nenumăratele specii, subspecii, varietăţi etc de peşti ornamentali care fac obiectul acvaristicii, incluzând aici chiar şi carasul auriu - *Carassius auratus auratus,* specie care este crescută pe alocuri în mici bazine artificiale în aer liber.

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THE EVALUATION OF CONSERVATION STATUS OF EURASIAN LYNX (*LYNX LYNX*) IN RODNA MOUNTAINS NATIONAL PARK (BIOSPHERE RESERVE), ROMANIA

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Abstract. The research is focused on assessment of conservation status of Eurasian lynx in Rodna Mountains National Park and surrounding areas from Eastern Carpathians (Romania). Combining different methods of assessment, such as footprints, droppings, video cameras with infrared sensors, the study concludes that the conservation status is good, the actual population being more than optimal.

Key words: Eurasian lynx, conservation, park.

Introduction

Rodna Mountains National Park (Biosphere Reserve) is one of the hot spots of Romanian and Carpathian biodiversity, having more than 7.500 flora and fauna species included in the official database. It also covers one of the oldest nature reserves from Romania – Pietrosu Mare (1932). The national park spreads on 47.000 ha and is placed in northern part of Romania, near the border with Ukraine, overlapping two counties – Maramureş and Bistriţa-Năsăud. There can be found all the mountainous ecosystems, the alpine belt being representative for Romania. Some habitats are well preserved; therefore, the area is the richest in endemic species (Iuşan, 2011).

From biodiversity point of view, a very important group is represented by large carnivores (brown bear, wolf, lynx) and one of the key species chosen for active monitoring is the Eurasian lynx (*Lynx lynx*), an indicator of wilderness. In the Romanian legislation, the *Lynx lynx* species is a species of community interest (Annex 4A, Law 49/2011) that requires strict protection and a species whose conservation requires the designation of special areas of conservation (Annex 3, Law 49/2011). In international legislation the *Lynx lynx* species appears as a species whose protection status is of less concern (IUCN RED LIST, Least concern ver 3.1) and in the Habitats Directive 92/43 EEC it appears in Annexes 2 and 4, as a species of community interest. In the Red Book of vertebrate species of Romania it appears to have a vulnerable status. The research focused on evaluation of conservation status for Eurasian lynx was realized during 4 years (2011-2015) by a high number of rangers, biologists, volunteers and students. The area for research is including the National Park.

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Materials and Methods

The methods used for assessing the conservation status of Eurasian lynx in Rodna Mountains are: footprints on snow, droppings, counting dead animals killed by lynx, mounting video cameras with infrared sensors during 4 years. The devices used for realizing the distribution map of lynx were: GPS units, photo cameras, Toughbook, GIS software, video cameras with infrared sensors, binoculars, field guides of footprints. Most of the footprints were marked in the winter season. More than 19 valleys from Rodna Mountains were mapped using GPS units and taking into account the footprint distribution.

Individual traces were recorded. The traces of lynx are like those of wild cat, but aproximately 3 times higher. The front print has about 6.5 cm in length and 5.5 cm in width and the back print has about 7.5 cm and 6 cm (MacDonald & Barett, 2005).

Signs of feeding: the lynx method to kill prey seems pretty typical. Salt on the animal's back and trying to kill their prey with an accurate bite on the neck. The bite on the neck can often be confirmed by the throat and the tracheae' wounds. In some cases, fine holes caused by the sharp canines can easily be seen if the wound is open. The distance between the canines is 25-33 mm. If the lynx is not able to reach the throat of the prey, neck bite occasionally but never bites the back. The marks left there can often be seen in wolf attacks. There are rarely signs of a struggle where lynx have killed prey (MacDonald & Barett, 2005).

Droppings: lynx feces resemble those of the cat, but are much larger. The droppings contain remains of feathers, fur and bones. Like wild cats, lynx cover their excrement with earth or snow (MacDonald & Barett, 2005).

The lynx monitoring method using video cameras represents an easy collection of information on species distribution, with little impact upon it. We used 10 video cameras with infrared sensors (Cam Bushnell Terophy model) for recording at night, with motion sensors, meaning that recording starts only when there is movement within the range of the sensor.

Video camera locations were chosen based on certain criteria: nature ecosystems with low human impact, ecosystems with high naturalness (old forests, no logging, no sheepfolds), low degree of habitat fragmentation, presence of lynx previously mentioned, presence of substantial animal traces. Due to these issues, it proved to be useful the use of surface lattice model. The surface of Rodna Mountains was devided in squares of 4.5 km^2.

The checking of the video cameras was performed every 2-3 weeks. During each check, the memory cards were downloaded to a laptop and the batteries were changed, if necessary. The cameras were set to record footage for 10-15 seconds, with a pause of 5 seconds between shots and with a resolution between 5-8 megapixels. Records were stored in a database.

Results and Discussions

The spatial distribution of Eurasian lynx (*Lynx lynx*) in Rodna Mountains National Park was also analysed using the observations collected from 9 hunting associations (Note de fax, 2003-2013), 18 forestry districts and also traces and droppings observed in the field between 2010-2015, by the Park Administration and volunteers (Fig. 1).

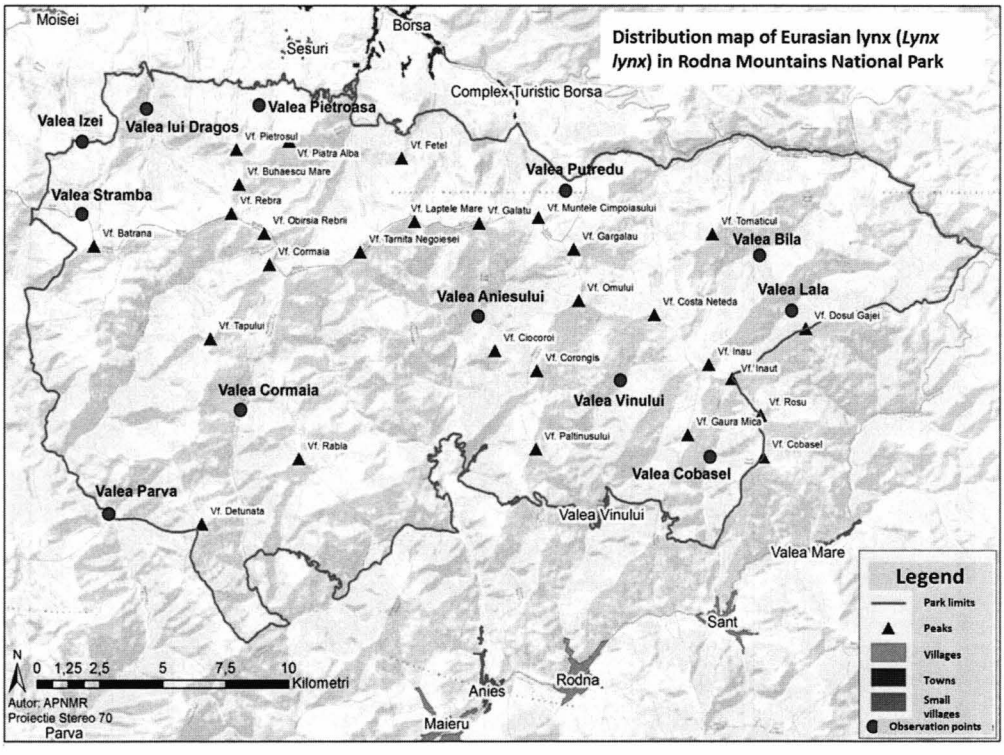

Fig. 1. The distribution map of Eurasian lynx (*Lynx lynx*) in Rodna Mountains National Park (Romania)

The individual territory depends on disponibility of the food, the lynx population density, the shelters met. A research performed between 2001-2004 on habitats used by lynx in the Eastern Carpathians showed that during summer, the minimal teritory of a female with cubs is around 2.500 ha (25 km^2) of forest (Predoiu, 2011). Individual territory in the Carpathians occupy 10 to 26 km^2 (Botnariuc & Tatole, 2005, Geacu, 2007, Kossak, 1988). In nocturnal trips for food, they can travel distances of 20-30 km, where prey is scarce. They used to cover the entire territory in 7- 10 days (Almăşan, 1989).

Based on population estimates, there can be seen the population dynamics of lynx in Rodna Mountains National Park. As shown in figure 2, the number of lynx within Rodna Mountains National Park evolved between 2003-

2013. The fluctuations show a significant increase from 27 lynxes (2003) to 42 lynxes in 2013. One reason for this performance we think it would be explained by reducing the acts of poaching and increasing the control factors, due to the establishment of Rodna Mountains National Park Administration (13 rangers), in 2004, at the same time with the enforcement of environmental laws in partnership with the Environmental Guard, the Moutain Gendarmerie.

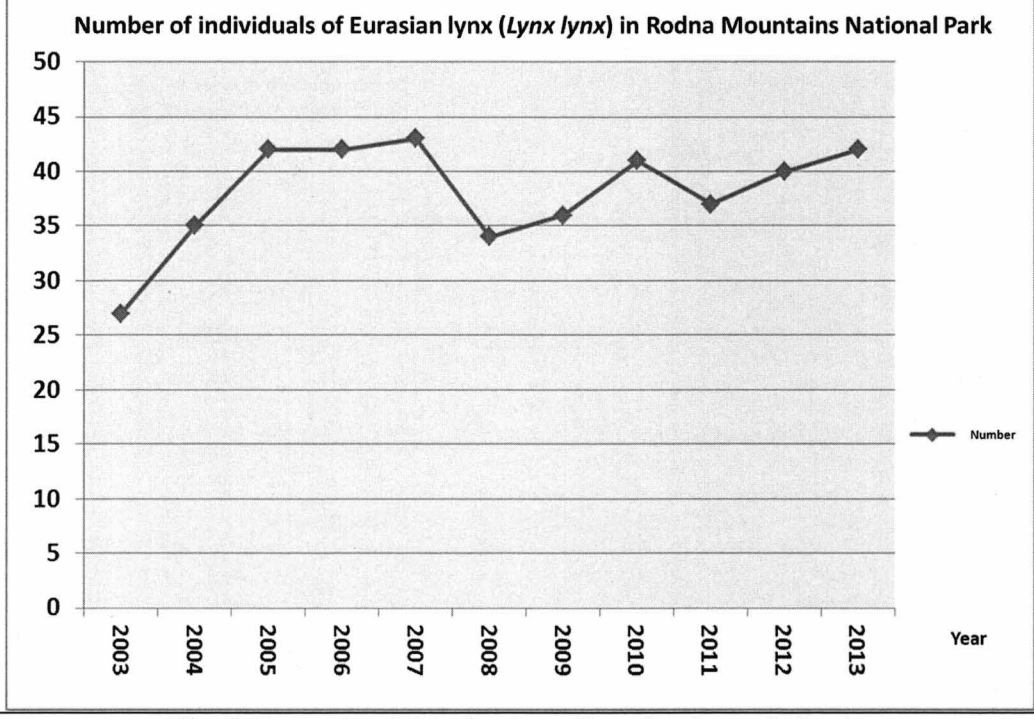

Fig. 2. Dynamic of Eurasian lynx (*Lynx lynx*) population in Rodna Mountains National Park

On the basis of applying the key diagnostics developed by ICAS and Transylvania University in 2002 and based on the research conducted in study areas in the Eastern Carpathians, it was developed a methodology for the characterization of habitats for lynx. With several significant changes made to the original model, we obtained a classification of habitats frequented by lynx from Rodna Mountains National Park (Table 1, Fig. 3).

On the basis of the practical aspects of wildlife management activities, as well as specific requirements related to implementing a conservative management of the lynx population, it is proposed the following classification of hunting plots in which lynx specimens are located:

- Class I land - refuge areas (areas essential for conservation);
- Category II lands - buffer zones (areas of transition and connection);
- Category III lands - risk areas (areas unfavorable).

Refuge areas for species conservation are key areas where most specific factors related to the existence of lynx have values above the national average. These areas are characterized by high densities of prey species and lynx, as well as anthropogenic habitat and favorable conditions for maintaining viable populations of lynx in the medium and long term. It is recommended that these areas be maintained and expanded, considering that, by implementing specific measures, the local population of lynx can be maintained in the medium and long term.

Buffer zones are areas of transition to less favorable conditions, with the role of connection. These areas are characterized by medium densities of prey species and lynx, as well as anthropogenic habitat and favorable conditions for maintaining the lynx population especially in the medium term, there are concerns relating to the maintenance of local lynx populations on long-term. These areas are particularly important to ensure connectivity and maintain exchange genes between viable populations of lynx uniformly distributed within the Carpathians, requiring specific management measures.

Risk areas present unfavorable conditions, not allowing the maintaining of lynx populations rather to a minimum scale, in the short term. These areas are characterized by low densities of prey species and lynx, as well as habitats and anthropogenic conditions unfavorable for maintaining medium-term lynx populations. The level of uncertainty related to developments in the next period is particularly high at local level, and management measures are mutch better in order to improve the existing situation. These areas have a high dependence versus anthropogenic factors and a delicate balance in natural ecosystems constantly subjected to significant pressure (Predoiu, 2011).

Table 1. Classification of habitats for lynx by frequence of presence in Rodna Mountains

No.	Place	Total points	Type of habitat
1.	Valea Anieşului	8.1	Refuge area
2.	Valea Cormaia	8	Refuge area
3.	Valea Cobăşel	9	Refuge area
4.	Valea lui Dragoş	12	Refuge area
5.	Valea Putredu	5	Buffer area
6.	Valea Strâmba	3	Risk area
7.	Valea Pietroasa	5	Buffer area
8.	Valea Parva	9	Refuge area
9.	Valea Vinului	7	Refuge area
10.	Valea Lala	8	Refuge area
11.	Valea Bila	9	Refuge area
12.	Valea Izei/Izbuc	5	Buffer area

Based on the scores obtained, we obtained a classification of refuge habitat areas for lynx such as: Cobăşel, Anieş, Cormaia, Izvoru Dragoş, Parva, Vinului, Lala valleys and a series of buffering zones such as: Putredu, Pietrosu, Iza Izbuc. Another category is the risk areas, as Strâmba Valley. The delimitation of Strâmba Valley as risk area is justified by the existence of numerous acts of poaching and intense hunting activities in the nearby of the national park boundaries (Fig. 3).

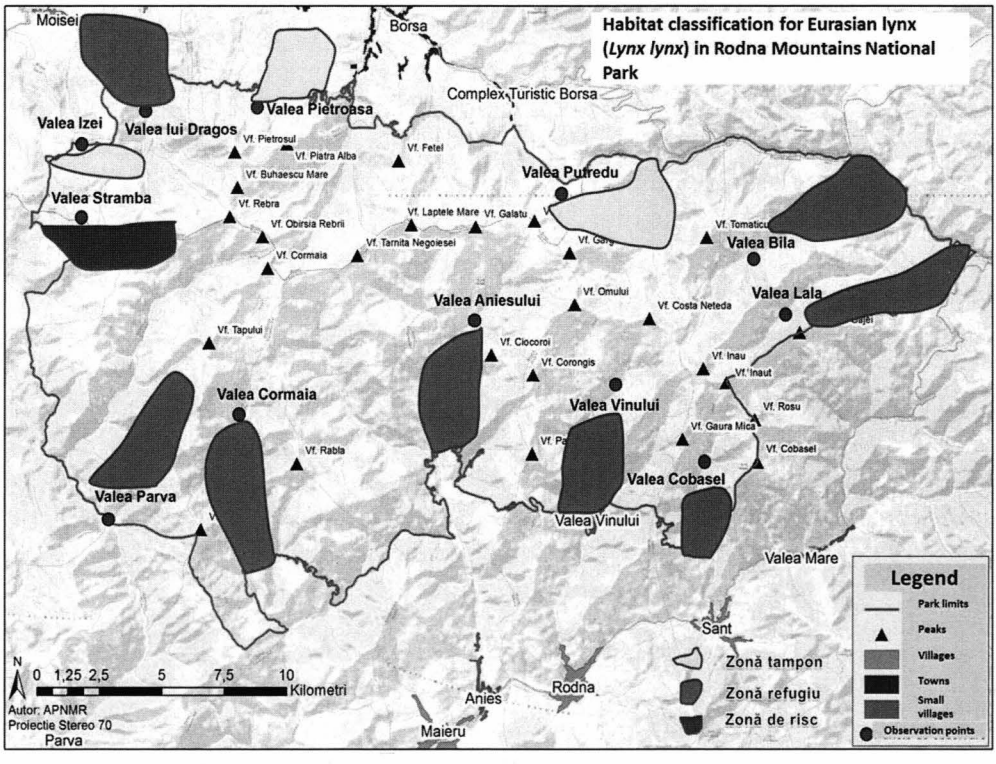

Fig. 3. Habitat classification for Eurasian lynx (*Lynx lynx*) in Rodna Mountains National Park

Regarding the estimation of optimal population, given that for a female with cubs is required a minimum area of 2.500 ha (Predoiu, 2011, Lehmkuhl, 1984, Iuşan, 2013), based on an estimated area of Rodna Mountains, it can be estimated a maximal number of 19 lynx females with cubs, plus a maximal of 10 males per number of females. For the total of 19 females estimated to be having 1 cub each (38 individuals) and 10 males, that means 48 individuals.

The minimal number that would exist in Rodna Mountains can be calculated due to the fact that lynx can cover an area of 11.000 ha (Predoiu, 2011, Thomas, 1990, Vasiliu, 1964) and corelating to the surface of Rodna Mountains, it results about 5 females of lynxes with cubs, that means 10 individuals, sumarizing 15 individuals.

The optimum population of the Eurasian lynx in Rodna Mountains was estimated by calculating an average value between the minimal and the maximal number of individuals. Due to the fact that in Rodna Mountains there is a minimal number of 15 individuals and a maximal of 48 individuals and the average population is estimated at a value of 31.5 individuals. We must take into account that the research was developed on a bigger area than the National Park, covering more than 100.000 ha.

Conclusions

In the period between 2010-2015 it was developed a process of assessing the Eurasian lynx (*Lynx lynx*) population from Rodna Mountains National Park and surroundings by the National Park Administration and volunteers. The results are showing a good conservation status of lynx in Rodna Mountains.

The study aimed to estimate the distribution map of lynx, there was identified the dynamic of population which depicts a rigorous population (42 individuals). Also was proposed a habitat classification and identifying the risk area for lynx (Strâmba Valley) and other refuge and buffer zones. There was estimated the maximum population (48 individuals), minimum population (15 individuals) and optimum population (32 individuals).

Rezumat. Cercetarea se axează pe evaluarea stării de conservare a râsului (*Lynx lynx*) în Parcul Naţional Munţii Rodnei şi împrejurimile din Carpaţii Orientali (România). Combinând diverse metode pentru evaluarea stării de conservare precum observarea urmelor, excrementelor, lăsăturilor, imaginile surprinse cu ajutorul camerelor video cu senzori în infraroşu de mişcare, s-a ajuns la concluzia că starea de conservare a speciei este bună, iar populaţia locală este peste optimul speciei.

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HEAVY METALS CONTAMINATION OF LETTUCE (*LACTUCA SATIVA*)

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Abstract. The excessive use of chemicals in agriculture leads to the accumulation in soil, groundwater and food chain of substances such as heavy metals, nitrites, nitrates, pesticides. For leafy vegetables, their contamination occurs not only by absorption, but also by contact with the superterranean, parts of the plants. The content of 4 heavy metals (Pb, Cd, Cu and Zn) was determined in three locations: one with historical contamination, other with contamination due to car traffic and an uncontaminated control zone. The content of heavy metals in lettuce samples studied shows a low contamination with cadmium, which is below the detection limit for lettuce grown in Cluj and Jucu; samples from Cluj-Napoca hawed the lowest content of zinc. Samples of lettuce in Jucu location (the witness) have the lowest values for the content of heavy metals, except zinc; the tendency was a decrease in three years. Samples from Şeica Mare location (zone with historical contamination) is distinguished by maximum contamination with heavy metals.

Key words: heavy metals, AAS, lettuce, contamination.

Introduction

Lettuce is grown for its leaves that are eaten raw or cooked. Being a plant resistant to cold and having a short period of vegetation is grown in successive and associated field and protected in greenhouses, solariums and tunnels, providing lettuce consumption staggered throughout the year. The importance of lettuce as food plant derives from its relatively high sugar content (2-3.5%), protein (1-1.6%), vitamins B1 (0.07 mg/ 100 g), B2 (0.12 mg/ 100 g), C (5-20 mg/ 100 g, phosphorus salts (1-7 mg/ 100 g) and potassium salts (260 mg/ 100 g) (Muntean et al., 2014). As a result of increased food needs, an increase of production in agriculture is aimed for all groups of crop plants. Intensive agriculture should be practiced with caution; approaches exaggerated, in disagreement with plant requirements associated with the appearance of serious problems of environmental pollution.

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Excessive use of chemicals in agriculture lead to impaired balance and soil accumulation in soil, groundwater and food chains of dangerous chemicals such as heavy metals, nitrites, nitrates, pesticides (Gavrilescu, 2009; McLaughin et al., 1996).

Pollution affects environmental quality and significantly affect plants as toxic dose depending on the plant, its stage of development, the nature of the contaminant, its concentration in the soil, the soil particularities and environmental conditions; leaf vegetables are able to accumulate heavy metals more than the roots, causing contamination thereof in the case of the absorption of not only the soil but also the contaminants contact with aerial parts of the plants (Alexander et al, 2006; Fu et al., 2009; Memon and Schröder, 2009; Uzu et al., 2010; Yang et al., 2009).

Intensive farming requires the use of chemical fertilizers, but fertilizer used not only contain the necessary macro elements for plant growth and development, but also heavy metal contaminants such as cadmium, lead, chromium, copper, zinc, nickel or mercury, thus creating the sources of soil contamination with heavy metals (Jarup, 2003; Mortvedt et al., 1981). As a result, the soils in which the fertilizers are also used in real time become deposits for heavy metals (Alloway et al., 1988).

Toxic effects of heavy metals in the tissues and plant cells vary depending on the concentration. Heavy metals have a high affinity for carboxyl and sulfhydryl groups, depending on their physico-chemical properties, such interactions result in inhibition of enzyme activity. An important effect shown for some metals as mercury, cobalt, cadmium, zinc is inhibiting chlorophyll pigments to *Phasoelus vulgaris* (Chaoui et al., 1997).

Agricultural and forest ecosystems of historical polluted areas, such as Copsa Mica, Zlatna or Baia Mare were deeply affected by pollution, soils in these areas therefore falling fertility in the lower classes.

Previous studies have revealed various levels of heavy metal contamination as follows: lettuce, 17-33 μg/kg Cd, 2-118 μg/kg Pb, potatoes, 12-26 μg/kg Cd 50-155 μg/kg Pb, beans, 1-3 μg/kg Cd, 2-59 μg/kg Pb (Samsøe-Petersen et al., 2002).

Materials and Methods

The experimental cultivated area is located near the central area of the Cluj-Napoca, coordinates 46 ° 45'56,77 'north latitude and 23 ° 34'0,64' east longitude (Fig. 1). This area is considered to be polluted due to car traffic.

Fig. 1. Satellite image of the experimental parcel's location from Cluj-Napoca (source: Google Earth)

Jucu location is at a distance of 20 km NE of Cluj-Napoca, at the crossroads of two major relief units: Somes Plateau in the west and in the east Transylvania Plain. The experimental field belongs to the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, having the coordinates 46°52'16 " N, 23°45'27" (Fig. 2). This lot is considered a control one.

Fig. 2. Satellite image of the experimental parcel's location from Jucu (source: Google Earth)

Şeica Mare is located in the southern part of the Transylvanian Depression; the northern boundary of the area is marked by Blaj Transylvania Plateau in the west of Plateau Secaşelor Amnas and the southern boundary is marked by Plateau Vurpar Hârtibaciului, near the valley of the Târnava Mare river corridor in most developed portion width with individual terraces and meadows well. In a relatively short distance are the city of Copşa Mică (known for the main polluter SC Sometra SA) and most important urban center in the valley Târnava Mare-Mediaş. The experimental area's coordinates are 46°01'51 N and 24°09'40" E longitude (Fig. 3).

Fig. 3. Satellite image of the experimental parcel's location from Şeica Mare (source: Google Earth)

In view of the determination of heavy metals, analytical techniques can be divided into methods for determining the sequence of the elements, such as atomic absorption spectrometry (flame variants (FAAS) or graphite furnace (GFAAS) - in which the analysis is carried out prior to the tests in solution) and methods for the simultaneous determination of several elements, as well as coupled plasma mass spectrometry (ICP-MS) or X-ray fluorescence spectrometry. The choice of method is based on the sensitivity, precision, accuracy and costs.

Atomic absorption spectrometry is used with a wide application not only for the determination of heavy metals, but also of other elements. In atomic absorption spectrophotometry, the samples are mineralized and brought into solution, then sprayed into the flame absorption spectrophotometry or atomized in a graphite furnace, depending on the configuration of the instrument. Measurements were performed using a double beam spectrophotometer Shimadzu AA-6300 (Shimadzu Corporation, Japan) with both flame atomizer and graphite furnace, equipped with deuterium lamp for background correction and hollow cathode lamps for the studied elements (Fig. 4); to increase the productivity of the measurements, the configuration included a autosampler ASC-6100F.

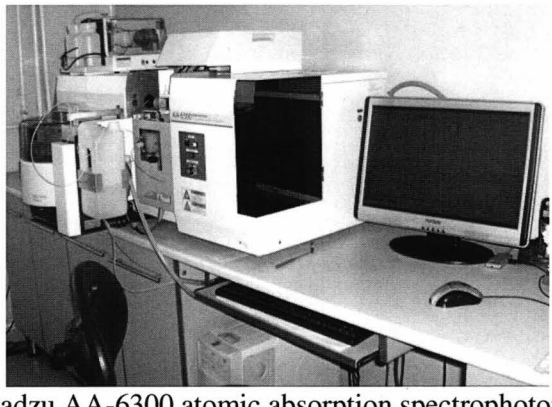

Fig. 4. The Shimadzu AA-6300 atomic absorption spectrophotometer utilized for heavy metals' determination

In order to assess the degree of recovery of heavy metals from the products studied, a series of samples were fortified with solutions of known concentrations of these metals. The achieved recovery levels are: 91.14% of Cd, 93.52% for Pb, 94 92% for Cu and 95.02% for Zn. The repeatability was determined based on 10 determinations made by the same operator keeping the same experimental conditions; yielded 3.87% relative standard deviation for lead, 2.63% for cadmium, zinc and 2.09 to 2.41 for copper.

The limits of detection (LOD) and quantification limits were calculated using the relationship:

$$LD = 3SD/slope \qquad LC = 10\ SD/slope$$

where: SD - standard deviation of 11 readings blank

slope - the slope of the calibration curve

The detection limits were as follows: 6 mg/ kg for copper, 6 mg/ kg for zinc, 0.18 μg/kg for lead and 0.04 μg/kg for cadmium.

Results

The content of heavy metals in lettuce samples studied shows a low contamination with cadmium, which is below the detection limit for lettuce grown in Cluj and Jucu (Tables 1 and 2); the samples from Cluj-Napoca have the lowest content of zinc (Table 1).

Samples of lettuce in Jucu location shows the lowest values for the content of heavy metals, except zinc; the tendency was for a decrease in the three years (Table 2). Samples of lettuce from Seica Mare location is distinguished by maximum contamination with heavy metals (Table 3).

Table 1. Average content of heavy metals from lettuce samples from Cluj-Napoca

Metal	UM	2012	2013	2014	Average	Standard deviation
Pb	μ/kg	0,06	**0,09**	0,04	**0,06**	0,03
Cd	μg/kg	**< 0,036**	**< 0,036**	**< 0,036**	-	-
Cu	mg/kg	**0,24**	0,22	0,15	**0,20**	0,05
Zn	mg/kg	1,83	**2,16**	1,71	**1,90**	0,23

Table 2. Average content of heavy metals from lettuce samples from Jucu

Metal	UM	2012	2013	2014	Average	Standard deviation
Pb	μg/kg	**0,03**	0,02	0,01	**0,02**	0,01
Cd	μg/kg	< 0,036	< 0,036	< 0,036	-	-
Cu	mg/kg	**0,18**	0,16	0,13	**0,16**	0,03
Zn	mg/kg	1,56	**2,49**	1,95	**2,00**	0,47

Table 3. Average content of heavy metals from lettuce samples from Şeica Mare

Metal	UM	2012	2013	2014	Average	Standard deviation
Pb	µg/kg	**1,41**	1,32	1,08	**1,27**	0,17
Cd	µg/kg	0,09	**0,14**	0,05	**0,09**	0,05
Cu	mg/kg	**2,96**	2,77	2,40	**2,71**	0,28
Zn	mg/kg	5,09	**9,45**	4,63	**6,39**	2,66

Conclusions

Data collected in the 2012-2014 period for determining the factors of influence of heavy metal contamination lead to the following conclusion.

• Road traffic is a significant factor influencing the contamination with heavy metals, by the emission of exhaust gases for lead, residual pollution due to use during the gasoline additive tetraethyl lead and by depositing metal particles released by vehicle movement (active pollution source for copper) and braking process by abrasion of tires (active pollution source for zinc);

• Industrial emissions: for heavy metals analyzed have at least one active source of pollution, influence manifested most strongly at Şeica Mare location, situated near Copşa Mică;

• Residual pollution: both Cluj-Napoca and Copşa Mică (located near the village of Şeica Mare) in recent decades have been characterized by a strong industrial activity recognized in the literature as polluting heavy metals (Copşa Mică was declared "ecological disaster zone").

Rezumat. Chimizarea în exces a agriculturii duce la tulburarea echilibrului solului şi la acumularea în sol, în apa freatică şi în lanţurile trofice a unor substanţe (metale grele, nitriţi, nitraţi, pesticide). Legumele consumate pentru frunze au capacitatea de acumulare a metalelor grele superioară celor cu rădăcini, în cazul acestora contaminarea producându-se nu doar prin absorbţia din sol, ci şi prin contactul contaminanţilor cu părţile aeriene ale plantelor. S-a determinat comparativ conţinutul de 4 metale grele (Pb, Cd, Cu si Zn) din trei locaţii: una cu contaminare istorică, una cu contaminare datorată traficului auto şi o a treia locaţie, zona nepoluată – considerată zonă martor. Conţinutul de metale grele în probele de salată studiate evidenţiază o contaminare redusă cu cadmiu, aceasta fiind sub limita de detecţie pentru salata cultivată în Cluj-Napoca şi Jucu; probele din Cluj-Napoca au cel mai scăzut conţinut de zinc. Probele de salată din locaţia Jucu (zona martor) prezintă cele mai scăzute valori pentru conţinutul de metale grele, cu excepţia zincului; se remarcă tendinţa de scădere a acestora în cei trei ani. Probele de salată din locaţia Şeica Mare (zona cu contaminare istorică) se remarcă prin contaminarea maximă cu metale grele.

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SOME EMERGING AND RE-EMERGING INFECTIOUS DISEASES

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Abstract. Many infectious diseases remain among the leading causes of death and disability worldwide for three reasons: emergence of new infectious diseases; re-emergence of old infectious diseases; and persistence of intractable infectious diseases. The problem of emerging infectious disease has recently captured the attention of the scientific community. New infectious diseases continue to evolve and emerge. Changes in human demographics, behavior, land use etc., are contributing to new disease emergence by changing transmission dynamics to bring people into closer and more frequent contact with pathogens. In addition to the continual discovery of new human pathogens, old infectious disease enemies are re-emerging. Natural genetic variations, recombination, and adaptations allow new strains of known pathogens to appear to which the immune system has not been previously exposed and is therefore not primed to recognize. Furthermore, human behavior plays an important role in re-emergence. Increased and sometimes imprudent use of antimicrobial drugs and pesticides has led to the development of resistant pathogens, allowing many diseases that were formerly easy treatable with drugs to make a comeback. Moreover, many important infectious diseases have never been adequately controlled epidemiological on either the national or international level. Infectious diseases that have posed ongoing health problems in developing countries are re-emerging in some other world parts.

Key words: emerging and re-emerging infections, infectious diseases.

Introduction

As definition if the disease was unknown in the location before, the disease is considered to be *emerging*. However, if the disease had been present at the location in the past and was considered eradicated or controlled, but have come back in a different form or a different location, the disease is considered to be *re-emerging* (Lederberg, 2000). The basic situation of an emerging or re-emerging infectious disease is their incidence which has increased in a defined time period and location (CDC, 1994, Morens et al., 2004). Multiple factors, including: economic development, land traditions/ behaviors, human demographics, international travel and commerce, contribute to the emergence and re-emergence of infectious diseases (Fauci et Morens, 2012).

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The emerging and re-emerging infections presentation, reveal the evolutionary properties of some pathogenic microorganisms and the dynamic relationships between them, their hosts and the environment (Morse, 1995).

1. ANTHRAX

Is an acute disease caused by the bacterium *Bacillus anthracis*. Occupational exposure to infected animals or their products (such as skin, wool, and meat), is the usual pathway of exposure for humans. Workers who are exposed to dead animals and animal products are at the highest risk, especially in countries where anthrax is more common. Most forms of the disease are lethal, and it affects both humans and other animals. Anthrax does not spread directly from one infected person to another and it is spread by spores. These spores can be transported by clothing or shoes. Because of their long lifespan, spores are present globally and remain at the burial sites of animals killed by anthrax for many decades. Until the 20th century, anthrax infections killed hundreds of thousands of animals and people worldwide each year (Fauci, 2003). The production of two powerful lethal toxins by the bacteria causes death (Lederberg, 2000). Anthrax toxin is a mixture of three protein components: protective antigen (PA), edema factor (EF), and lethal factor (LF). PA plus LF produces lethal toxin, and PA plus EF produces edema toxin. These toxins cause death and tissue swelling respectively. Anthrax can enter the human body through the intestines (ingestion), lungs (inhalation), or skin (Figure 1) and causes distinct clinical symptoms based on its site of entry (Morse, 1995). Effective vaccines against anthrax are available, and there is even an antibiotic treatment for the disease. Prevention: early detection of sources of anthrax infection can allow preventive measures to be taken.

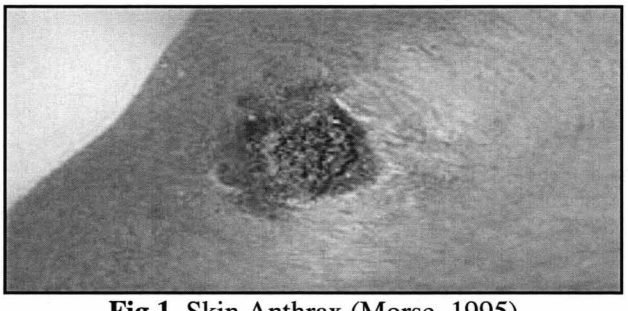


Fig.1. Skin Anthrax (Morse, 1995)

2. FLU INFECTION

Flu activity is high across most of the country with flu illnesses, hospitalizations and deaths elevated situations. Flu season will probably continue for several weeks in each yar. There are many different influenza A viruses, some are found in humans and others in animals such as avian flu in birds and poultry.

Disease. Influenza (flu) is a contagious respiratory illness caused by influenza viruses. It can cause mild to severe illness. Serious outcomes of flu infection can result in hospitalization or death. Some people, such as older people, young children, and people with certain health conditions, are at high risk for serious flu complications. It can cause mild to severe illness, and at times can lead to death. The flu is different from a cold. The flu usually comes on suddenly. People who have the flu often feel some or all of these ***symptoms:*** fever or feeling feverish/chills, cough, sore throat runny or stuffy nose, muscle or body aches, headaches, fatigue (tiredness). Some people may have vomiting and diarrhea, though this is more common in children than adults Most people who get influenza will recover in a few days to less than two weeks, but some people will develop complications (such as pneumonia) as a result of the flu, some of which can be life-threatening and result in death. Pneumonia, bronchitis, sinus and ear infections are examples of complications from flu. The flu can make chronic health problems worse. For example, people with asthma may experience asthma attacks while they have the flu, and people with chronic congestive heart failure may experience worsening of this condition that is triggered by the flu. The CDC Influenza Division International Program works with a wide range of international partners, including the World Health Organization, National Ministries of Health and others to build capacity to respond to pandemic influenza and to prevent and control seasonal influenza. All these activities include helping establish, expand, and maintain influenza surveillance and laboratory capacity, helping develop global and local pandemic plans and influenza prevention policies, supporting targeted research projects to address critical needs, and building the evidence base for decisions on influenza vaccine program expansion. Flu is highly infectious – but the annual flu jab can help to prevent it. Vaccination can still protect some people and reduce hospitalizations and deaths, and will protect against flu viruses (Deac, 2014). Influenza antiviral drugs can treat flu illness. CDC recommends these drugs be used to treat people who are very sick or who are at high risk of serious flu complications who have flu symptoms. Early antiviral treatment works best.

3. HEPATITIS

There are several kinds of Hepatitis, up to the virus who produce them: Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus (Table 1), Hepatitis E virus, Hepatittis G virus (WHO 2014, Deac 2014).

Hepatitis Viruses					
	HAV	HEV	HBV	HCV	HDV (only in combination with HBV)
Symptoms	anorexia,malaise,dark urine,pale stools,vomiting,headache,jaundice				
Transmission	Enteric		Parenteral		
Classification	Picornavirus	Hepevirus	Hepadnavirus	Flavivirus	Deltavirus
Viral genome	ssRNA	ssRNA	dsDNA	ssRNA	-ssRNA(negative sense)
Viral antigens			HBsAg HBeAg	Core antigen	Delta antigen
Incubation	15-45 days	15-60 days	4 months(45-160d)	2 months(15-150d)	1-2 months
Chronic Hepatitis	No	No	Yes 10% of acute infections	Yes >50% of acute infections	Yes <5 % of coinfections 80% of superinfections

lifehugger

Table 1. Most important Hepatitis viruses and their characteristics (WHO 2014, Deac 2014)

The transmission of viruses can be realized by infected body fluids in Hepatitis B, or via food and water as in Hepatitis A and E, or can only be contracted if it exist already Hepatitis B infection. The most common from all Hepatitis infection is Hepatitis A. Notices about this viral infection and it's are different to other type of infections (Fauci, 2003) and this make the disease relevant (Figure 2).

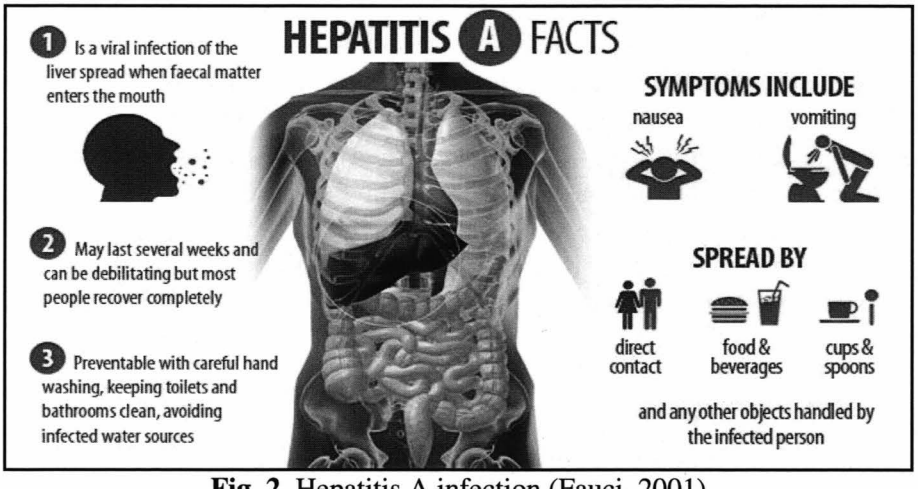


Fig. 2. Hepatitis A infection (Fauci, 2001)

Hepatitis B and C cause most health problems and even life complications or death causes for human beings. Hepatitis B is a liver disease caused by the hepatitis B. HBV can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death. HBV is spread in healthcare settings when blood or other body fluid from an infected person enters the body

of a person who is not infected. In a healthcare setting, this contact is primarily through contaminated needles, syringes, or other sharp instruments. Hepatitis C is a liver disease (Fauci, 2001) caused by the hepatitis C virus (HCV). HCV can cause lifelong infection, cirrhosis, or liver cancer (Figure 3).

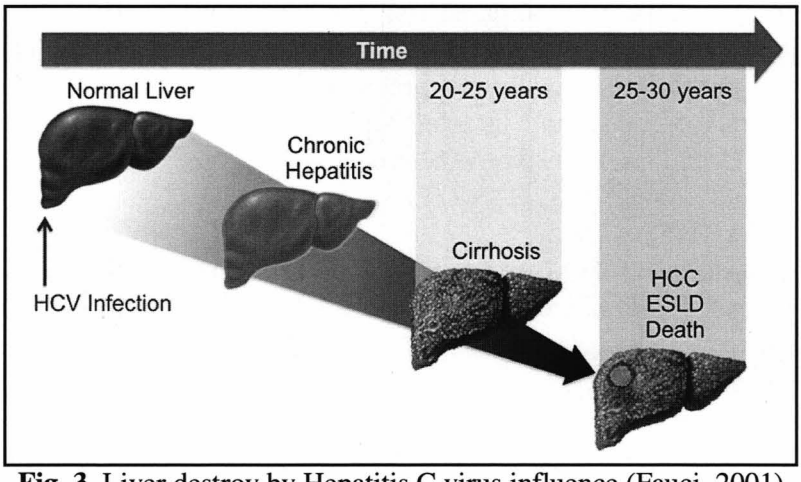


Fig. 3. Liver destroy by Hepatitis C virus influence (Fauci, 2001)

HCV is spread by contact with the blood of an infected person (Figure 4). The spread of HCV from one person to another in healthcare settings is rare, but can occur (Deac, 2014, Fauci, 2003). In a healthcare setting, this contact is primarily through contaminated needles, syringes, or other sharp instruments.

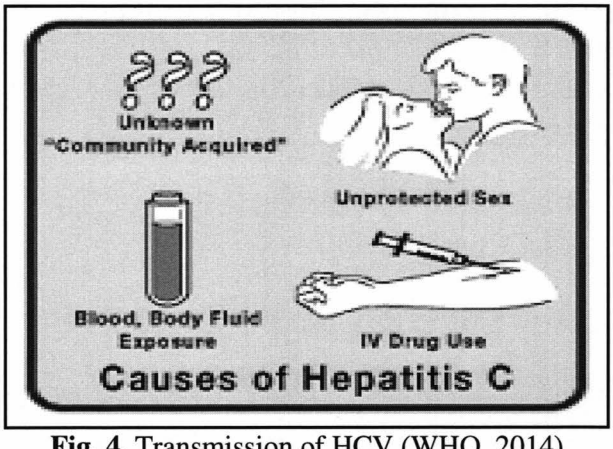


Fig. 4. Transmission of HCV (WHO, 2014)

Prevention of Hepatitis B and C. Hepatitis B virus is largely preventable through vaccination. Outbreaks of HBV and HCV infection have occurred in outpatient settings, hem dialysis units, long-term-care facilities, and hospitals, primarily as a result of unsafe injection practices; reuse of needles, finger stick devices, and syringes; and other lapses in infection control. To

prevent transmission of blood borne pathogens, health care workers should adhere to recommended standard precautions (Deac, 2014) and fundamental infection-control principles, including safe injection practices and appropriate aseptic techniques). For continued protection, the Advisory Committee on Immunization Practices (ACIP) recommends that health care and public safety workers with reasonably anticipated risk for exposures to blood or infectious body fluids receive the complete Hepatitis vaccination, which is the best prevention against Hepatitis B. No precautions are recommended for HCV, because the risk of becoming infected and passing the infection on to others after an exposure to HCV is low (Morens et al., 2004).

4. PLAGUE

It is a disease that affects humans and other mammals. It is caused by the bacterium, *Yersinia pestis*. There are known, 3 plague outbreaks during the times. Humans usually get plague after being bitten by a rodent flea that is carrying the plague bacterium or by handling an animal infected with plague. Plague is infamous for killing millions of people in Europe during the Middle Ages. Plague has a remarkable place in history and has had enormous effects on the development of modern civilization (Krause, 1992). For centuries, plague represented disaster for people living in Asia, Africa and Europe and because the cause of plague was unknown, plague outbreaks contributed to massive panic in cities and countries where it appeared. Numerous references in art, literature and monuments attest to the horrors and devastation of past plague epidemics. Today, modern antibiotics are effective in treating plague. Without prompt treatment, the disease can cause serious illness or death. Presently (Figure 5), human plague infections continue to occur in the western United States, but significantly more cases occur in parts of Africa and Asia (Garrett, 1994).

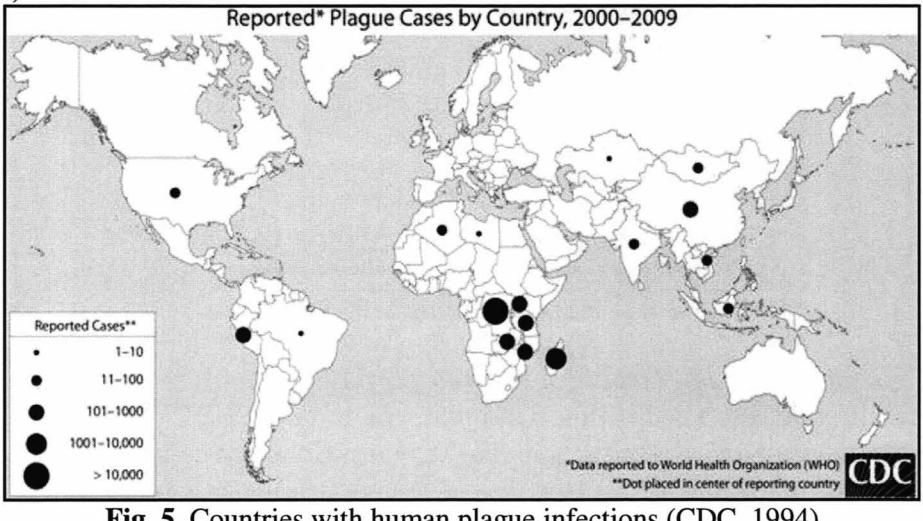
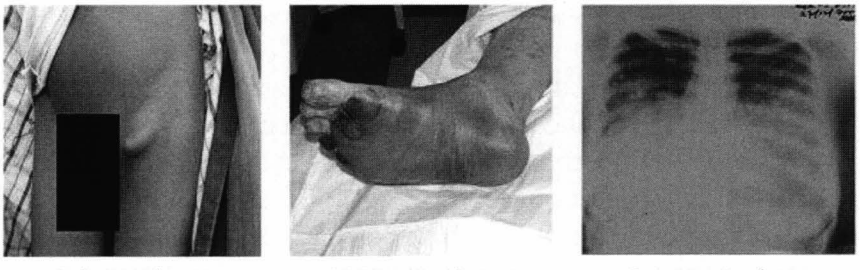


Fig. 5. Countries with human plague infections (CDC, 1994)

Transmission. In urban areas or places with dense rat infestations, the plague bacteria can cycle between rats and their fleas. The plague bacteria can be transmitted to humans in the following ways. *Flea bit.* Plague bacteria are most often transmitted by the bite of an infected flea. During plague epizootics, many rodents die, causing hungry fleas to seek other sources of blood. People and animals that visit places where rodents have recently died from plague are at risk of being infected from flea bites. Dogs and cats may also bring plague-infected fleas into the home. Flea bite exposure may result in primary – *Bubonic plague or septicemic plague; Contact with contaminated fluid or tiss*ue**,** when humans can become infected when handling tissue or body fluids of a plague-infected animal; *Infectious droplets*-when a person has plague pneumonia, they may cough droplets containing the plague bacteria into air who breathed in by another person and can cause *pneumonic plague*. This type of spread has not been documented in the United States since 1924, but still occurs with some frequency in developing countries. Cats are particularly susceptible to plague, and can be infected by eating infected rodents.

Plague symptoms depend on how the patient was exposed to the plague bacteria and can take different clinical forms (Figure 6), but the most common are bubonic, pneumonic and septicemia (Krause, 1992). Plague is a serious illness. Prompt ***treatment*** with the correct medications is critical to prevent complications or death**.** The most common sign of bubonic plague is the rapid development of a swollen and painful lymph gland called a *bubo* (Garret, 1994).



Bubonic plague Septicemic plague Pneumonic plague

Fig. 6. Clinical forms of Plague infection (Garret, 1994)

Diagnosis is made by taking samples from the patient, especially blood or part of a swollen lymph gland, and submitting them for laboratory testing.

Prevention. The best prevention measures are: reduce rodent habitat around home, work place, and recreational areas. Remove brush, rock piles, junk, cluttered firewood and possible rodent food supplies, such as pet and wild animal food. Wear gloves for potentially infected animals to prevent contact between skin and the plague. Keep fleas off of pets by applying flea control products. Animals that roam freely are more likely to come in contact with

plague infected animals or fleas and could bring them into homes. That for do not allow dogs or cats that roam free in endemic areas or to sleep on your bed.

5. EBOLA

Until I wrote this article (end of Jan. 2015), the ***statistical situation*** for this disease were: *Total Cases: 21408*; *Laboratory-Confirmed Cases: 13510; Total Deaths: 8483.* Ebola viruses were last year found (WHO, 2014) in several African countries (Figure 7).

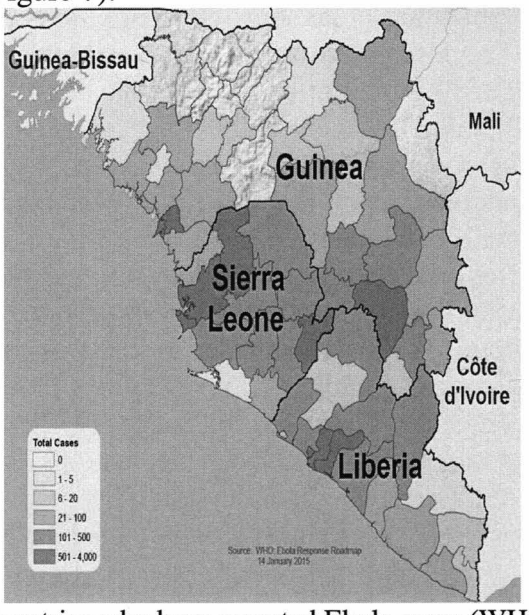


Fig. 7. Countries who have reported Ebola cases (WHO, 2014)

The disease was first discovered in 1976 near the Ebola River in what is now the Democratic Republic of the Congo. Since then, outbreaks of Ebola among humans have appeared sporadically in Africa. Because the natural reservoir host of Ebola viruses has not yet been identified, the way in which the virus first appears in a human at the start of an outbreak is unknown.

Transmission. However, scientists believe that the first patient becomes infected through contact with an infected animal, such as a fruit bat or primate (apes and monkeys), which is called a spillover event (Peters, 2005). Person-to-person transmission follows and can lead to large numbers of affected people. In some past Ebola outbreaks, primates were also affected by Ebola and multiple spillover events occurred when people touched or ate infected primates.

When an infection occurs in humans, the virus can be spread to others through direct contact (through broken skin or mucous membranes in, for example, the eyes, nose, or mouth) with: blood or body fluids (including but not limited to urine, saliva, sweat, feces, vomit, breast milk, and semen) of a person who is sick with Ebola, objects (like needles and syringes) that have been

contaminated with the virus, infected fruit bats or primates (apes and monkeys). Ebola is not spread through the air, by water, or in general, by food. However, in Africa, Ebola may be spread as a result of handling bush meat (wild animals hunted for food) and contact with infected bats. There is no evidence that mosquitoes or other insects can transmit Ebola virus. Only a few species of mammals (e.g., humans, bats, monkeys, and apes) have shown the ability to become infected with and spread Ebola virus. Healthcare providers caring for Ebola patients and family and friends in close contact with Ebola patients are at the highest risk of getting sick because they may come in contact with infected blood or body fluids (Peters, 2005). During outbreaks of Ebola, the disease can spread quickly within healthcare settings, where hospital staff is not wearing appropriate personal protective equipment.

Symptoms includes: fever, severe headache, muscle pains, weakness, fatigue, diarrhea, vomiting and abdominal (stomach) pain, unexplained hemorrhage (bleeding or bruising). Symptoms may appear anywhere from 2 to 21 days after exposure to Ebola, but the average is 8 to 10 days.

Recovery from Ebola depends on good supportive clinical care and the patient's immune response. People who recover from Ebola infection develop antibodies that last for at least 10 years.

Prevention. Healthcare workers who may be exposed to people with Ebola should follow these steps: wear appropriate personal protective equipment (PPE), practice proper infection control and sterilization measures. It had to be isolate patients with Ebola from other patients, or of whom have died from. The virus can enter the body through broken skin or unprotected mucous membranes in, for example, the eyes, nose, or mouth.

Infection control is a key strategy in stopping the spread of Ebola and identifying and managing patients with the Ebola virus. In an area affected by an Ebola outbreak, make sure to do the following: practice careful hygiene (wash your hands with soap and water or an alcohol-based hand sanitizer); avoid contact with blood and body fluids; do not handle items that may have come in contact with an infected person's blood or body fluids (such as clothes, bedding, needles, and medical equipment); avoid funeral or burial rituals that require handling the body of someone who has died from Ebola; avoid contact with bats and nonhuman primates or blood, fluids, and raw meat prepared from these animals; avoid facilities in West Africa where Ebola patients are being treated. After the return from Africa, it had to monitor the health for 21 days and do seek medical care immediately if developing Ebola disease symptoms (Peters, 2005). Dedicated medical equipment, preferably disposable, when possible (WHO, 2014, Fauci 2003), should be used by healthcare personnel providing patient care (Figure 8).

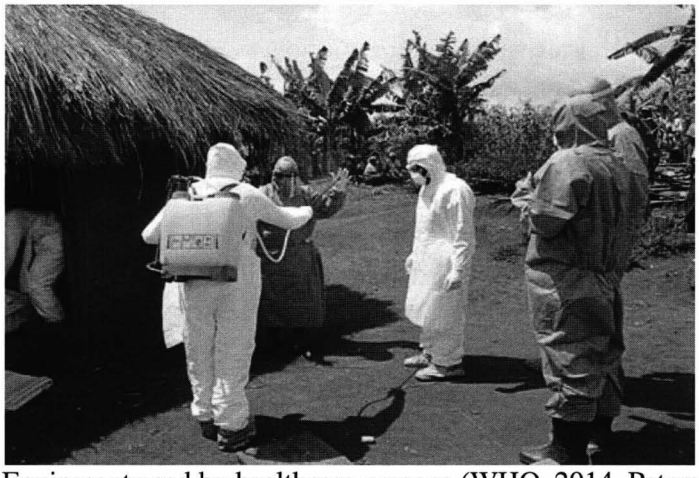


Fig. 8. Equipment used by healthcare persons (WHO, 2014, Peters, 2005)

As a ***precaution***, men who have recovered from Ebola are advised to abstain from sex (including oral sex) for three months. If abstinence is not possible, it has to be used condoms.

6. HIV and AIDS

General Information. Human immunodeficiency virus (HIV) is the virus that can lead to Acquired Immune Deficiency Syndrome (AIDS). HIV destroys blood cells called CD4+ T cells, which are crucial to helping the body fight disease. This results in a weakened immune system, making persons with HIV or AIDS at risk for many different types of infections (Fauci, 2003). Transmission of HIV to patients while in healthcare settings is rare. However, proper sterilization and disinfection procedures are required to prevent infection risks. Most exposures do not result in infection. Up to CDC data, more than 35 million people worldwide are living with HIV (WHO, 2014, Morens et al., 2008).

Although HIV transmission in healthcare settings, it is extremely rare. Medical experts emphasize that the careful practice of infection control procedures because of the universal precautions (i.e., using protective practices and personal protective equipment to prevent transmission of HIV and other blood borne infections) who protect patients as well as healthcare providers from possible HIV transmission in medical and dental settings. Important factors that influence the overall risk for occupational exposures to blood borne pathogens include the number of infected individuals in the patient population and the type and number of blood contacts. CDC has documented rare cases of patients contracting HIV in healthcare settings from infected donor tissue. Although the risk factors for HIV are the same for everyone (Figure 9), some racial/ethnic groups are far more affected than others (WHO, 2014, Fauci, 2003). Because the most common ways HIV is transmitted is through anal or

vaginal sex, or sharing drug injection equipment with a person infected with HIV, it is important to take steps to reduce the risks associated with these. Some gender groups are far more affected by HIV infection than others. Gay, bisexual, and other men who have sex with men, for example, account for the majority of new infections despite making up only 2% of the population (Fauci, 2003, Taylor et al., 2001).

Fig. 9. Several transmissions for HIV (Taylor et al., 2001)

Prevention and Control of HIV. To be tested for HIV antibody as soon as possible after exposure (base line) and periodically for at least 6 months after the exposure (e.g., at 6 weeks, 12 weeks and 6 months). If you take antiviral drugs for post exposure treatment, you should be checked for drug toxicity by having a complete blood count and kidney and liver function tests just before starting treatment and 2 weeks after starting treatment. It must be reported any sudden or severe flu-like illness that occurs during the follow-up period, especially if it involves fever, rash, muscle aches, tiredness, malaise, or swollen glands. Any of these may suggest HIV infection, drug reaction, or other medical conditions. CDC has provided funding to HIV partners to help implement programs that will help curb the increase of HIV infections. These programs facilitated with our partners and grantees are critical in the goal of eliminating HIV infection in the world (WHO, 2014, Taylor et al., 2001).

7. TUBERCULOSIS

It is caused by *Mycobacterium tuberculosis*. ***Transmission*** of *Mycobacterium tuberculosis* is a recognized risk to patients and healthcare personnel. Transmission is most likely to occur from patients who have unrecognized pulmonary tuberculosis or tuberculosis related to their larynx, are not on effective anti-tuberculosis therapy, and have not been placed in tuberculosis isolation. Transmission in Healthcare Settings has been associated with close contact with persons who have infectious tuberculosis, particularly during the performance of cough-inducing procedures such as bronchoscopy and sputum induction. *Mycobacterium tuberculosis* is spread through air and

can travel long distances. Cases of multidrug-resistant tuberculosis (MDR-TB, which includes extensively drug-resistant tuberculosis [XDR-TB]), have been recognized and are more difficult to treat. The bacteria usually attack the lungs, but TB bacteria can attack any part of the body such as the kidney, spine, and brain. If not treated properly, TB disease can be fatal. TB is spread through the air from one person to another. The TB bacteria are put into the air when a person with TB disease of the lungs or throat coughs, sneezes, speaks, or sings (Figure 10). People nearby may breathe in these bacteria and become infected. Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist: latent TB infection and TB disease (WHO, 2014).

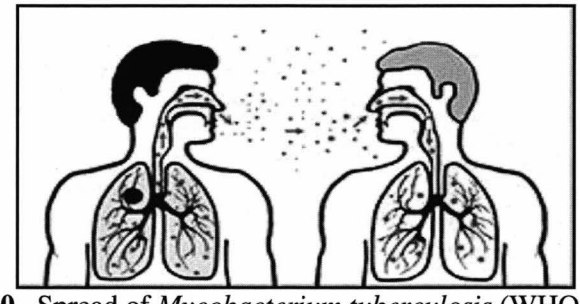


Fig. 10. Spread of *Mycobacterium tuberculosis* (WHO, 2014)

Latent TB Infection. TB bacteria can live in the body without making you sick. This is called latent TB infection. In most people who breathe in TB bacteria and become infected, the body is able to fight the bacteria to stop them from growing. People with latent TB infection do not feel sick and do not have any symptoms. People with latent TB infection are not infectious and cannot spread TB bacteria to others. However, if TB bacteria become active in the body and multiply, the person will go from having latent TB infection to being sick with TB disease. TB Disease. TB bacteria become active if the immune system can't stop them from growing. When TB bacteria are active (multiplying in your body), this is called TB disease. People with TB disease are sick. They may also be able to spread the bacteria to people they spend time with every day. Many people who have latent TB infection never develop TB disease. Some people develop TB disease soon after becoming infected (within weeks) before their immune system can fight the TB bacteria. Other people may get sick years later when their immune system becomes weak for another reason. For people whose immune systems are weak, especially those with HIV infection, the risk of developing TB disease is much higher than for people with normal immune systems. Symptoms of TB disease include: a bad cough that lasts 3 weeks or longer, pain in the chest, coughing up blood or sputum, weakness or fatigue, weight loss, no appetite, chills, fever, sweating at night.

Testing for TB Infection. There are two kinds of tests that are used to detect TB bacteria in the body: the TB skin test (TST) and TB blood tests.

These tests can be given by a health care provider or local health department. If you have a positive reaction to either of the tests, you will be given other tests to see if you have latent TB infection or TB disease. There are two kinds of tests that are used to detect TB bacteria in the body: the TB skin test (TST) and TB blood tests. These tests can be given by a health care provider or local health department. If you have a positive reaction to either of the tests, you will be given other tests to see if you have latent TB infection or TB disease.

Tuberculosis prevention and control. It can be particularly problematic in correctional and detention facilities, in which persons from diverse backgrounds and communities are housed in close proximity for varying periods. This report provides a framework and general guidelines for effective prevention and control of TB in jails, prisons, and other correctional and detention facilities. Recommendations were developed on the basis of published guidelines and a review of the scientific literature. Effective TB-prevention and -control measures in correctional facilities include early identification of persons with TB disease through entry and periodic follow-up screening; successful treatment of TB disease and latent TB infection; appropriate use of airborne precautions (e.g.,airborne infection isolation, environmental controls, and respiratory protection); comprehensive discharge planning; and thorough and efficient contact investigation. These measures should be instituted in close collaboration with local or state health department TB-control programs and other key partners. Continuing education of inmates, detainees, and correctional facility staff is necessary to maximize cooperation and participation (Fauci and Morens, 2012, CDC., 1994). To ensure TB-prevention and -control measures are effective, periodic program evaluation should be conducted.

8. SALMONELLOSIS

Is an infection with bacteria called *Salmonella*. *Salmonella* germs have been known to cause illness for over 100 years (Morens et al., 2004). They were discovered by an American scientist named Salmon, for whom they are named. There are many different kinds of *Salmonella* bacteria. *Salmonella* serotype *typhimurium* and Salmonella serotype *Enteritidis* are the most common in the United States (WHO, 2014). Salmonellosis is more common in the summer than winter. Children are the most likely to get salmonellosis. The rate of diagnosed infections in children less than five years old is higher than the rate in all other persons. Young children, the elderly and the immunocompromised are the most likely to have severe infections. It is estimated that approximately 400 persons die each year with acute salmonellosis. *Salmonella* live in the intestinal tracts of humans and other animals, including birds. It is usually *transmitted* to humans by eating foods contaminated with animal feces. Contaminated foods usually look and smell normal. Contaminated foods are

often of animal origin, such as beef, poultry, milk, or eggs, but any food, including vegetables, may become contaminated. Thorough cooking kills *Salmonella*. Food may also become contaminated by the hands of an infected food handler who did not wash hands with soap after using the bathroom. *Salmonella* may also be found in the feces of some pets, especially those with diarrhea, and people can become infected by. Reptiles, such as turtles, lizards, and snakes, are particularly likely to harbor *Salmonella*. Many chicks and young birds carry *Salmonella* in their feces. People should always wash their hands immediately after handling an animal, even if it is healthy one (Deac, 2014).

The illness. Most persons infected with *Salmonella* develop: diarrhea, fever, and abdominal cramps, 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment, or only with rehydration. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness. Once *Salmonella* has been identified, further testing can determine its specific type. Antibiotic therapy can prolong the duration of excretion of *non-typhoidal Salmonella* and is recommended only for patients with severe illness.

Prevention. There is no vaccine to prevent salmonellosis. People who have salmonellosis should not prepare food or pour water for others until their diarrhea was not resolved. Many health departments require that restaurant workers with *Salmonella* infection have a stool test showing that they are no longer carrying the *Salmonella* bacterium before they return to work. People should wash their hands after contact with animal feces. Children can be exposed to the bacteria by simply holding, cuddling, or kissing the birds. Children should not handle baby chicks or other young birds. Improvements in farm animal hygiene, in slaughter plant practices, and in vegetable and fruit harvesting and packing operations may help prevent salmonellosis caused by contaminated foods. Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination and other food handling errors that can lead to outbreaks. Wider use of pasteurized egg in restaurants, hospitals, and nursing homes is an important prevention measure (Lederberg, 2000).

Special to be discussed from Salmonellosis desease is **TYPHOID FEVER**, which is a life-threatening illness caused by the bacterium *Salmonella typhi*. The disease is still common in the developing world, where it affects about 21.5 million persons each year (CDC, 1994, WHO, 2014). It can be prevented and can usually be treated with antibiotics. *Salmonella typh*i lives only in humans. Persons with typhoid fever carry the bacteria in their

bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever, but continue to carry the bacteria (Deac, 2014). Typhoid fever can be get by eating food or drink beverages that have been handled by a person who is shedding *Salmonella typhi* or if sewage contaminated with *Salmonella typhi* bacteria gets into the water used for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where hand washing is less frequent and water is likely to be contaminated with sewage. Once *Salmonella typhi* bacteria are eaten or drunk, they multiply and spread into the bloodstream. The body reacts with fever and other signs and symptoms. Some basic actions can *prevent* from Typhoid fever, as: avoid risky foods and drinks; avoid foods and beverages from street vendors; hands must be washed carefully with soap and water after using the bathroom, and there is not allowed to prepare or serve food for other people. It can be used vaccination against Typhoid fever. Even if the symptoms seem to go away, it may still be carrying *Salmonella typhi*. If so, the illness could return, or could pass the disease to other people. In fact, if such person works at a job where it had to handle food or care for small children, the person may be barred legally from going back to work, until is no longer carry any typhoid bacteria (WHO, 2014).

Rezumat. În lume multe boli infecţioase rămân lidere ca şi cauze de deces sau dizabilităţi create, din trei motive: emergenţa unor infecţii noi, reemergenţa unor infecţii mai vechi şi persistenţa potenţialului de contractare a unor boli infecţioase. Problema infecţiilor emergente a capturat atenţia comunităţilor ştiinţifice. Noi infecţii continuă să evolueze şi să emeargă azi. Schimbările demografice, obiceiuri şi tradiţii etc., contribuie la apariţia a noi boli emergente, prin schimbarea dinamicii transmiterii, prin apropierea oamenilor, sau prin mai mult contact cu patogenii. În adiţie cu noile descoperiri ale patogenilor umani, agenţi ai infecţiilor vechi, devin reemergenţi. Variaţiile genetice, recombinările şi adaptabilitatea lasă să apară noi tulpini patogene, faţă de care sistemul imun expus odinioară nu le mai poate recunoaşte. Mai departe şi obiceiurile joacă un rol important în apariţia bolilor reemergente. Astfel, creşterea chiar imprudentă a folosirii antibioticelor, a pesticidelor, a condus la dezvoltarea unor patogeni rezistenţi, permiţând multor boli să reapară, boli care înainte erau uşor tratabile. Trebuie remarcat şi că multe boli infecţioase nu au fost niciodată adecvat controlate epidemiologic, atât la nivel naţional sau internaţional. Aşadar bolile infecţioase care au creat probleme de sănătate în ţările dezvoltate devin acum reemergente în alte zone ale lumii.

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THE FOREST – A SOURCE OF RENEWABLE ENERGY

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Abstract. The value of fuel, wood or chopped straw depends on the caloric quantity of energy that can be recuperated. The quantity of recuperated thermal energy varies according to the content of humidity and the chemical composition of the wood.

In this paper it is our aim to evaluate the quality of wood in a biomass center that belongs to a wood processing plant in a mountainous environment in Romania. This is necessary as too much humidity in wood or in the wood biomass used for heating, reduces the output of the biomass furnace and leads to a fast wear of the heating system and subsequently to higher heating costs.

Key words: renewable resources, wood biomass, wood chips, storage center, wood waste, moisture/humidity.

Introduction

One of the most important challenges on national and international level is to ensure the local supply of ecological energy. The European Union has accepted this challenge and took concrete steps to put into practice a European package concerning climate and energy.

Biomass is a diversity of elements of vegetal origin in our surroundings and come from nature itself or as a result of human activity in order to advantageously exploit natural resources. We refer to their diversity existing in nature but also to their ability to permanently and rapidly regenerate. We now have the possibility to create from these resources value for ourselves. Biomass stores huge quantities of energy and is more and more used commercially. These energy sources are renewable, easy to store and have few negative aspects from the point of view of CO_2 emissions compared to other categories of biofuels (***Contract IEE/10/115/512.591387, 2011-proiect BiomassTradeCenters2).

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The content of moisture is a critical parameter with firewood. It is vital for a proper working of heating furnaces or heating systems as well as for the evaluation of the caloric value of a load of fuel (http://itermice.wordpress.com/2013/07/29/lemne-verziude-vs-lemne-uscate).

Most of the heating furnaces, which are run with wood, are designed to achieve a maximum of power using fuel having humidity in a limited range. The use of fuels that are outside said range will make the functioning inefficient and generate emissions.

The most common use of wood biomass to produce thermal energy is firewood (round wood with bark). In the last 10 years in Romania as well as elsewhere grew the quantity of the use of wood biomass in the form of pellets and briquettes mainly due to the legislation of environment protection through which companies are obliged to use wood waste. In 2011 almost 15% of the houses in towns and more than 60% in the country (almost 3 million of households in all) use wood biomass as a source of thermal energy (*** Contract IEE/10/115/512.591387, 2011-proiect BiomassTradeCenters2). According to recent estimations more than 2.4 million m^3 of wood (round wood in bark) is used to produce thermal energy only.

Biomass from forestry

Biomass from forestry represents a direct source of wood biomass from forests and other tree covered areas to produce energy – after woodworks, waste from tree cutting (top of trees, branches, bark, tree stumps), remains from arranging green zones (wooden biomass from parks, gardens, trimming of trees and hedges), dead wood (Berkesy et al., 2014).

It also represents an indirect source of wood biomass for the production of energy – waste from the trimming and processing of wood (bark, saw dust), secondary products from the cellulose and paper industry, processed fire wood, recycled wood after it was used (wood that was recycled in order to produce energy, wood waste from households.

The quality of firewood differs depending on the species of trees that are used for firewood, the conditions under which the trees grew, the shape and dimensions of particles, moisture of the wood, calorific value. The calorific value of wood depends on: moisture content -%- (mass of water/total mass) and density of wood (depends on tree species) - table 1.

Table 1. Calorific value of wood depending on moisture content

Wood condition	Moisture content	Net calorific value
Fresh wood	50-60%	2 kWh/kg
Store over one summer	25-35%	3,4 kWh/kg
Store over several years	15-255	4,0 kWh/kg

*** Contract IEE/07/054, 2008: *Wood Fuels Handbook*

Moisture is the most important factor that influences the quality of firewood and that determines its quality from the energetic point of view. The best efficiency from the energetic point of view is achieved with wood where the moisture is smaller than 20% (Table 1).

Hardwood dries more slowly compared to the wood that comes from species of softwood trees. The most slowly to dry is oak. To burn newly cut trees or wet wood means to get a small quantity of energy and it may lead to the damage the heating system. (*** Contract IEE/05/067/SI2.420197, 2008, Manual for firewood production, VTT-R-11021-08).

Dry wood is more efficient, it produces a great quantity of energy, the emissions into the atmosphere are reduced and it delivers more energy compared to wet wood.

The importance of wood moisture for heating

Freshly cut timber contains a great deal of water that may weigh up to one half of the cut timber. Not all the moisture in the wood is to be found in the tissues under the same conditions. A part of this water, namely one half of the total quantity that circulates in the leading and in the mixed tissues, is called free water. Less than 1% of the remaining water is in the walls of the vegetal tissues and is called hygroscopic water. The remaining water up to 1% is chemically combined in the elements that make the wood material. The chemically bound water cannot be dissolved but through the chemical discomposure of the wood (http://cooplan.ro/wp-content/uploads /2012/04 /2.6 - Wood).

If freshly cut wood is left under the influence of air, one can see that its moisture decreases slowly. First the free water evaporates and then the hygroscopic water until the equilibrium between the moisture of the wood equals the atmosphere moisture and remain constant (Table 2). In equilibrium the vapor tension of the wood is dependent on the partial pressure of vapor from the atmosphere. For the climate conditions in Romania, the relative humidity of dry wood in the open air varies between 12-15% (http://cooplan.ro/wp-content/uploads/2012/04/2.6.-Lemnul).

Table 2. Moisture of the wood compared to the raw wood* and to the dry wood**

Type of wood	U.M.	Absolute humidity **	Relative Humidity*
Pine	%	80-90	44-47
Spruce	%	80-100	44-50
Birch	%	60-80	37-44

http://cooplan.ro/wp-content/uploads/2012/04/2.6.-Lemnul)

Storage of wood that is to be used as firewood in specialized heating centers

In open wood biomass storage spaces, wood is stored and dried in the open air until humidity gets to 23%-35%. The store is organized as an open raw material store, where the firewood is stored in bulk in the open air in an uncovered sheds, split logs 1 m or 33 cm long are stored in boxes made of wood or metal, that facilitate (depending on the place – e.g. sunny places) the drying of the logs (Figure 1).

Wood chips are products that come from cutting the raw material and that after a storage period of about six months (spring-summer) in the covered part of the storage place, can be sold for use as firewood (Figure 1).

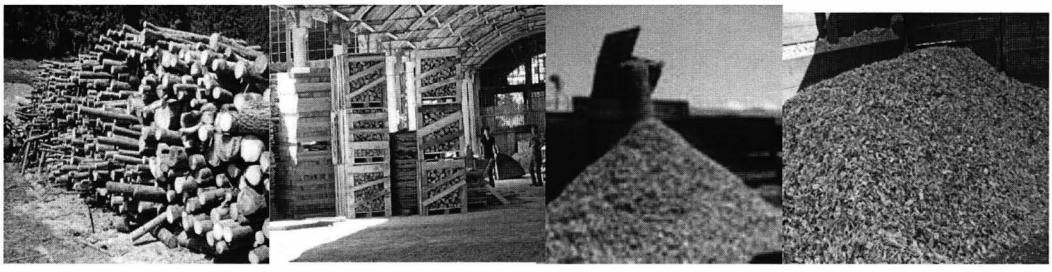


Fig. 1. Storage of firewood and wood chips in the biomass center

Material and method

In this study we intend to evaluate the quality of wood biomass that is to be used for the heating of living areas by means of thermal systems and therefore we analyzed firewood batches and wood chips in a storage place belonging to a wood processing company in the northern part of Romania.

The experiment consisted in the evaluation of the quality of the firewood of different wood species (beech, spruce) that were stored in different conditions within the storage place, taking into account the variation of moisture during the drying process in the open air in said area.

The firewood from the analyzed batches was cut in different periods of the year. The climate in the region varied during the time the test was carried out thus the drying of the firewood under natural conditions was different from this point of view, too.

We analyzed 6 batches of round firewood that was to be chopped. We chose two batches of wood (beech and spruce) that had been split and stored in a roofed court. The beech and spruce had been cut in December 2013 and December 2014 and was monitored for 11 months. Other two batches of wood, beech and conifer wood (spruce and fir) had been cut in January 2013 and 2014 and were monitored for 12 months; during this time the wood that had been cut to a length of 1000 mm was stored in a ventilated place. We also monitored 4 batches of wood that had been cut in December 2013 and December 2014 and

that was to be chipped after it had been dried. The respective wood was from beech and different species of conifers).

In order to evaluate the quality of the biomass taken to be analyzed we monitored the moisture parameter that was important for the storage process and for the use of the fuel and important in the use of firewood and wooden chips for the heating of different types of heating systems. To this scope we used the following standards:

- CEN/TS 15150:2005- Solid biofuels - Sampling
- EN 14774-3:2009 –Determination of moisture;

Results and discussions

What the natural drying of firewood concerns, this should be as dry as possible. We can get a good efficiency and a longer life of the furnace by using firewood that has been drying for two years. The heating power of the wood is inversely proportional to its humidity (http://www.centrale-termice.ro/prezentari-centrale-termice/cazane-pe-combustibil - solid-consideratii-generale).

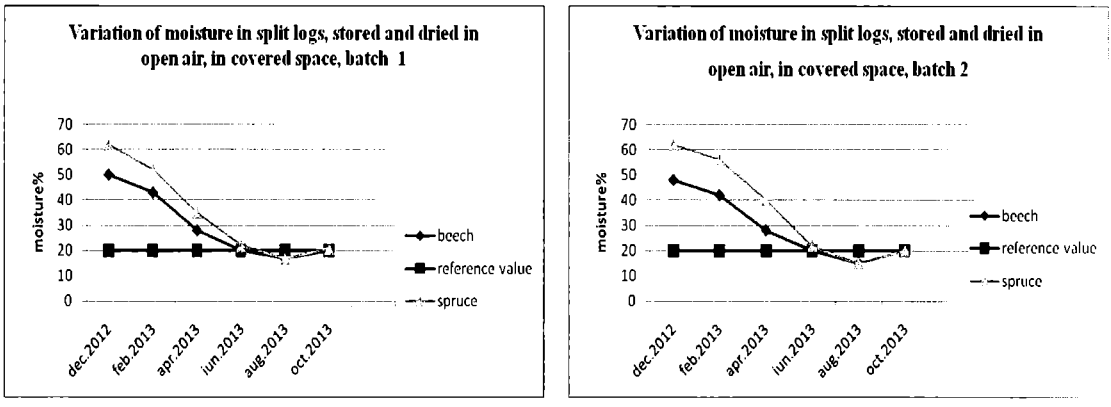


Fig. 2. Variation of moisture of firewood (split logs),, dried in open air and a covered area in 2013 (batch 1 and 2)

The moisture of freshly cut wood is about 55%, 60% and decreases depending on the drying conditions and on the wood species to 15-20% within several months or two years.

Logs of firewood lose humidity even beginning with winter should it be cut in the late fall. It loses the greatest deal of water in March.

In 2013 (Figure 2) the split and piled firewood was stored in a covered place. Having in mind that the summer 2013 was particularly hot and humid, the evolution of moisture was particularly good and the wood moisture was in June and the beginning of July 20% (M20), in the case of batch 1 (beech) and 22% (M20) with batch 2 (spruce).

When the moisture in summer is large with a lot of rain as it was in the summer of 2014 (Figure 3) the detectable differences are small and the value of moisture reaches 20 % a month later, i.e. at the beginning of August.

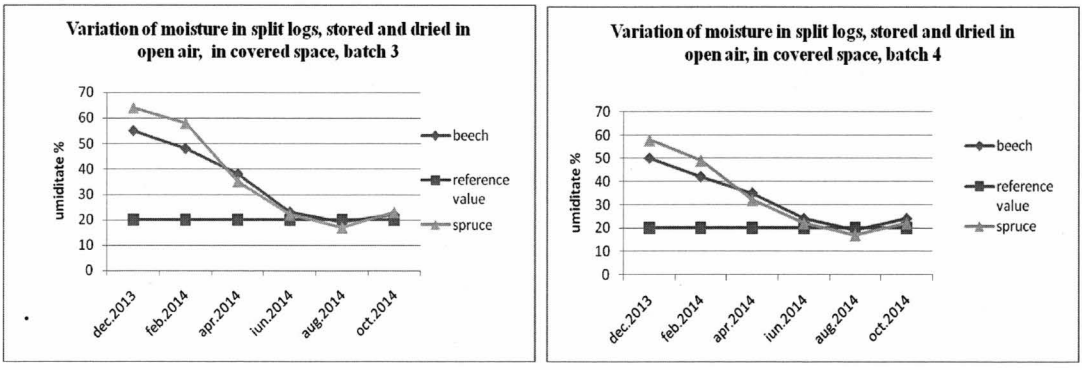


Fig. 3. Variation of moisture in firewood (split logs), dried and stored in open air, in covered space in 2014 (batch 3 and 4)

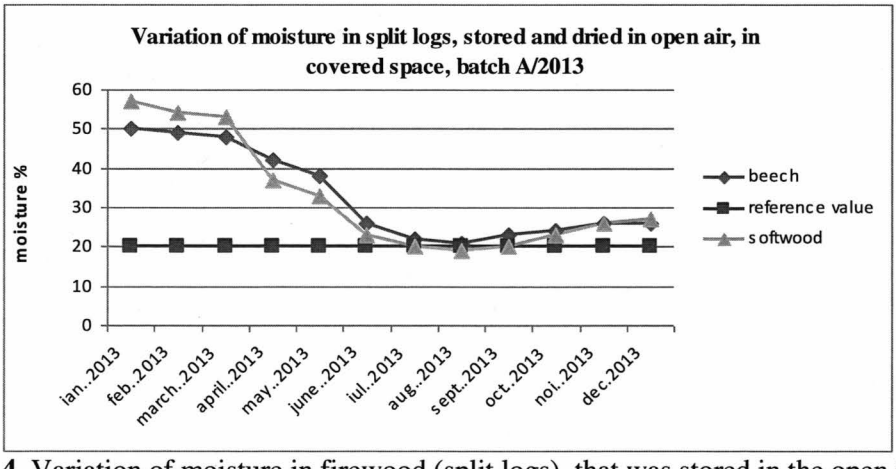


Fig. 4. Variation of moisture in firewood (split logs), that was stored in the open in a covered space 2013 (batch A)

Beginning in May spruce dries more rapid than beech, although the latter seems in the beginning drier because of the initial moisture that is smaller as well as because the loss of water was more rapid. In any case it takes the two species less or more the same time to get to M20. In April the evaporated quantity of water is at its highest (Figure 2, 3, 4, 5).

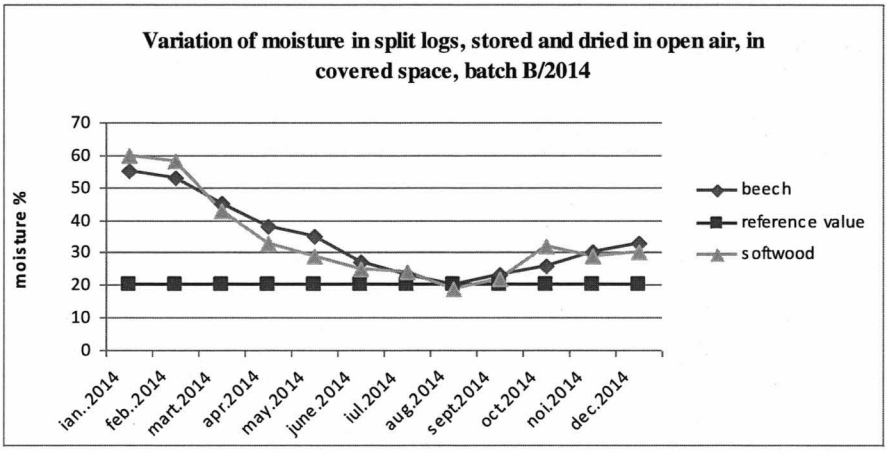
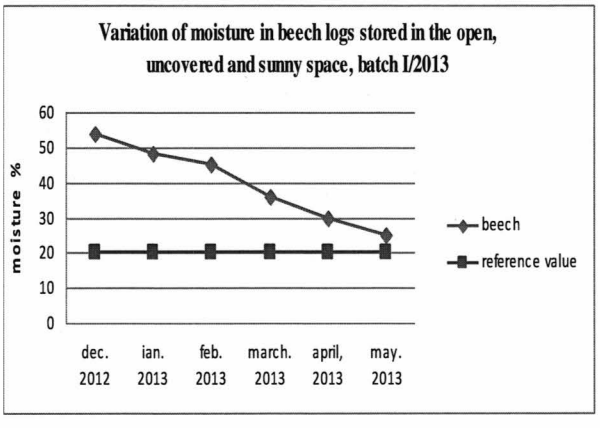


Fig. 5. Variation of moisture in firewood (split logs), that was stored in the open in a covered space 2014 (batch B)

Figure 4 and 5 show the variation of moisture with two batches of firewood with split logs of 1000 mm and stored in a covered and ventilated place, the ventilation of air stimulated the loss of water from the wood. The batches we analyzed were beech and conifers. The moisture decreased slower due to the dimensions of the wood, but at the beginning of July nevertheless it was of 22% (M20) with the beech and 20% (M20) with the conifers in batch A. In the following months until December moisture decreased to 21% in August (beech) and 19% (conifers), 26% (beech) and 27% (conifers) in December respectively. The surveillance of moisture showed a similar evolution for batch B (beech and conifers). Thus in July the moisture of the beech was 23% (M20) and 24% (M20), and reached in December 33% (M30) with beech and 30% (M30) with conifers respectively.



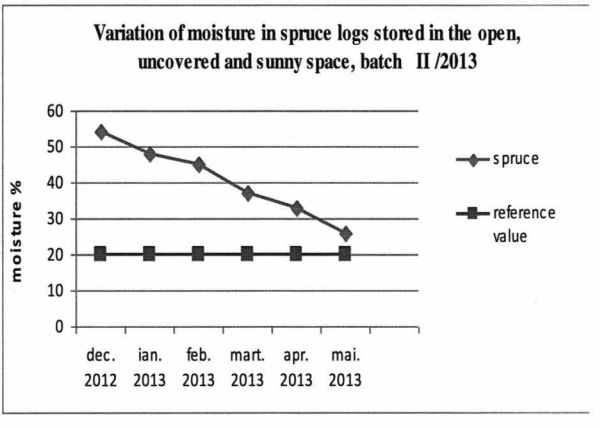


Fig. 6. Variation of moisture in dried firewood that was dried and stored in the open uncovered, sunny space 2013 (batch I and II)

The batches of beech and spruce, that were to be chopped, had a positive evolution concerning moisture. Thus batch I and II were kept in 2013 (January – May) in a sunny open place. Humidity decreased to 25% and 26% respectively and thus the wood was fit to be chopped (Figure 6).

The batches of beech and spruce of the batches III and IV were analyzed in 2014 (January – May) and had also been kept in a sunny, open place. Moisture decreased to 29% and 32% respectively, the moisture was thus larger than in the previous year and this can be explained by the fact that rains fell more often during this time (Figure 7).

Nevertheless the best storing technique was during the summer in sunny open places. A cover or a tarpaulin does not mean that moisture decreases in wood; they ensure protection of the dry wood from rain in order to avoid moisture to increase (Viser et al., 2014).

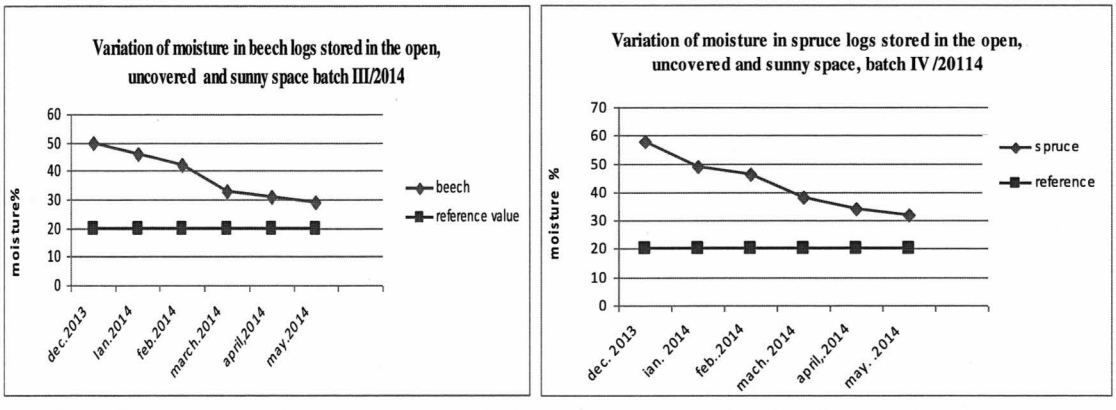


Fig. 7. Variation of moisture in dried firewood that was dried and stored in the open uncovered, sunny space 2014 (batch II and IV)

Conclusions

The firewood in the analyzed batches was harvested in different periods of the year and therefore the drying period is different, too. Climate and the specific characteristics of the seasons also play their parts with the variations of moisture of the stored firewood.

The drying of the wood has a series of positive aspects: it improves the storage system, it increases the energetic value, it decreases the transport weight, the quantity of ashes and of emissions into the atmosphere decrease, transport costs are reduced.

Experiments carried out during 2013 and 2014 showed that it takes several months to decrease the moisture in firewood if the logs are split and stored in covered, ventilated places; their evolution is very near to that of several wood species.

Whole logs kept in sunny places may take 5-6 months to reach moisture that is suitable to get chips ready to be sold.

Rezumat. Valoarea combustibilului, fie lemn sau tocătură, depinde de cantitatea de energie calorică ce poate fi recuperată. Cantitatea de energie termică recuperată variază funcție de conținutul de umiditate și compoziția chimică a materialului lemnos.

În lucrarea de față ne propunem să facem o evaluare a calității materialului lemnos dintr-un depozit de biomasă aferent unei fabrici de prelucrare a lemnului dintr-o zonă de munte a țării noastre. Acest lucru este necesar deoarece umiditatea prea mare a lemnului sau a biomasei de lemn folosită pentru încălzire reduce randamentul cazanelor de biomasă, ducând la deprecierea rapidă a acestora și totodată crește prețul pentru încălzirea spațiului de încălzit.

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http://cooplan.ro/wp-content/uploads/2012/04/2.6.-Lemnul)

http://www.centrale-termice.ro/prezentari-centrale-termice/cazane-pe-combustibil-solid-consideratii-generale

***EN 14774-3:2009 Solid biofuels - Determination of moisture content

*** Contract IEE/10/115/512.591387, 2011-proiect BiomassTradeCenters2

*** Contract IEE/07/054, 2008: Wood Fuels Handbook

*** Contract IEE/05/067/SI2.420197, 2008, Manual for firewood production, VTT-R-11021-08

*** CEN/TS 15150:2005- Solid biofuels - Sampling

THE EFFECTS OF MUSIC ON LEARNING AND MEMORY IN ADOLESCENTS

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Abstract. Studies have shown that music can affect memory and learning, but there are a number of factors that could influence the results. In our studies we left the "Mozart effect" which claims that listening to classical music can increase cognitive performance.

We wanted to examine the effect of background noise (in this case music) on short-term memory in adolescents taking into account that nowadays most young people say they study while listening to music, or if noise can influence in any way the ability to withhold certain information.

Within our project attended by 160 pupils aged 13 to 17 years. Students were divided into 4 groups of 40 students: 3 experimental groups and one control group.

The experiment consisted in applying two tests of visual memory and spatial ability pursuing to the students, in the presence of 3 music styles (hard-rock, classical and modern). The control group was tested in conditions of silence without any disturbing factor.

In the present study, we clearly demonstrated that the process of memorizing is hampered by disturbing factors, one of which in our case was music. It influenced subjects' ability to remember certain details of the images had to look carefully, while in a quiet environment this process occurs normally.

Key words: music, short memory, ability to recall information.

Introduction

Memory is the psychic imprint of the storage and updating of the information process. It is involved in the acquisition process of learning as experienced by the individual, an experience that will mediate adaptive responses to environmental stresses.

Throughout time many studies have been conducted to demonstrate how memories are formed and the factors that influence this process.

Memories are formed by processes of protein synthesis. Proteins directly related to forming memories are still unknown. The amount of synthesized proteins is correlated with the number of stimulus applications. Memories can be modified by more protein synthesis, which modifies existing synapses and also leading to the formation of new synapses.

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Therefore, short-term memories need less protein than in case off long-term memories (Kandel, 2001). Further studies have shown that some of the proteins used are already existing proteins (Hawkins et al., 2006).

Cognitive studies indicate that certain sounds and even music can influence the amount of information that an individual may remember (Bock et al., 2001). Other studies have been directed at the effect of music on memory according to different personality types.

Lately, psychologists focused on studying the effects of music on the brain, stressing the importance of musical memory on our behavior and emotional states. Music consists of sound, timbre, vibration, motion. To be enjoyable various intellectual and cultural symbolic components are added to the vibration of music. There are two main types of music: music for meditation, relaxing and dynamic music - rock, house, modern.

Studies about the impact of music on the human body are becoming more elaborate and highlight that exposure to sounds from earliest infancy increases memory and learning ability in children.

A group of Canadian researchers at McMaster University have observed two parallel groups of children aged 4 to 6 years. The first group made music classes for a year, the second group did not follow any music course. Tests carried out on two groups of children completed the study showed that the little that followed music lessons developed a greater ability to retain and juggle things easier with simple math concepts compared with those who did not attend music classes.

Another study conducted on a group of 12 children watched the brain activity during exposure to two types of sounds: One violin and one without any significance. The researchers found an increase in the intensity of brain function on the children who were exposed to the sound of the violin. Dr. Takako Fujioka, from Baycrest's Rotman Research Institute, one of the authors, supports the introduction of music education programs in preschool and school due to stimulation of cognitive development in children.

Studies have shown that music can affect memory and learning, but there are a number of factors that could influence the results. Many argued that the background music especially classical music can improve a person's cognitive performance and even memory (Stainbak et al., 1973). Positron emission tomography show that Mozart 's music activated 90-100% of the cerebral cortex, unlike each of their favorite. Generarly, symphonic and chamber music activates brain by 90 % as opposed to tango, which activate up to 50% from de brain. Best results have been registered in case of baroque music, whose sound waves have frequencies that fall within the optimal activation of brain activity.

In our studies we left the "Mozart effect" which claims that listening to classical music can increase cognitive performance. Other studies (Chabris, 1999 and Olsen 1995, 1997) refute Mozart effect and believe that whatever

their nature, noises may adversely affect intellectual performance. We wanted to examine the effect of background noise (music - in this case) on short term memory in adolescents (taking into account that nowadays most young people say that studying while listening to music), or to study if noise can influence in any way the ability to withhold certain information.

Materials and Methods

Within our project 160 pupils aged 13 to 17 years attended. Students were randomly chosen and four groups were formed: a control group and 3 experimental groups. Each experimental group consisted of 40 students each.

Each group of students in the experimental group underwent a genre (classical music, hard-rock and modern), while the control group was being tested in silence without any disturbing factor acting on them.

The experiment consisted in applying two tests that focused one the short time visual and spatial memory. In the first test, students were presented a collage of images consisting of 20 items. The image was displayed for 30 seconds. Then we asked the students to recall and write on a paper sheet as many of the items shown.

The second test consisted of 12 words display on the screen. Every word having 10 seconds display. The students were then asked to recall and write on a sheet of paper as many of the 12 words as possible.

The results were interpreted according to the age, the sex, respectively the habit of subjects to listening music in the background or not while learning. Correlations have been made between the results obtained.

All results were correlated and reported to the control group.

Results

Of the total participants in this study the largest group was represented by boys both in the experimental (over 73%) and the control group (57.14%) (Fig.1).

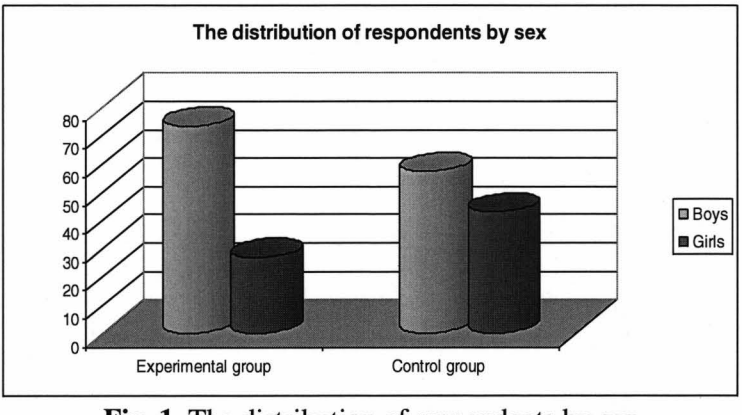

Fig. 1. The distribution of respondents by sex

The group tested in the presence of hard rock music was made up of students aged between 13-17 years old. Interpretations made according to age are shown in Figure 2. The results were expressed as a percentage. Thus, the total number of respondents to this musical style the most numerous categories was those of 17 years. It is usually encountered in a percentage of 28.26%.

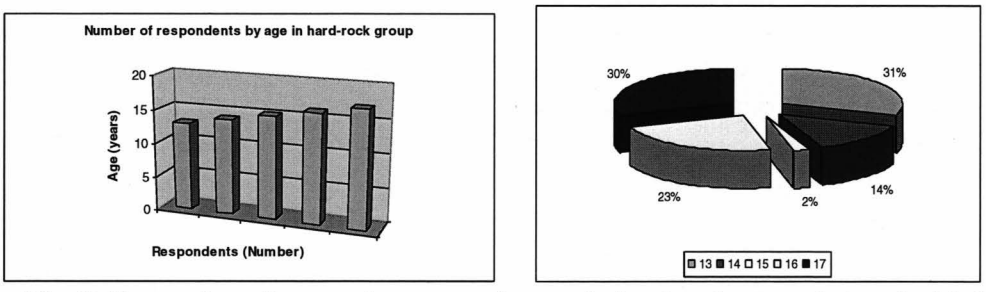

Fig. 2. Expression of respondents group by age in hard-rock group (numerical-left) (percentage - right)

Regarding the group tested in the presence of classical music their numerical expression by age is represented in Fig. 3. The most numerous respondents in this category belong to the group of 16 years.

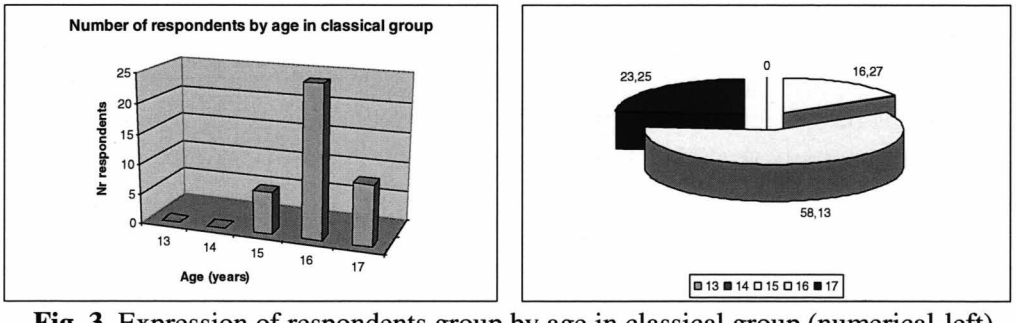

Fig. 3. Expression of respondents group by age in classical group (numerical-left) (percentage - right)

In the case of the third group tested in the presence of modern music were a total of 40 students. The distribution by age is shown in Fig. 4. The largest group in this case was between the ages of 15 and 16.

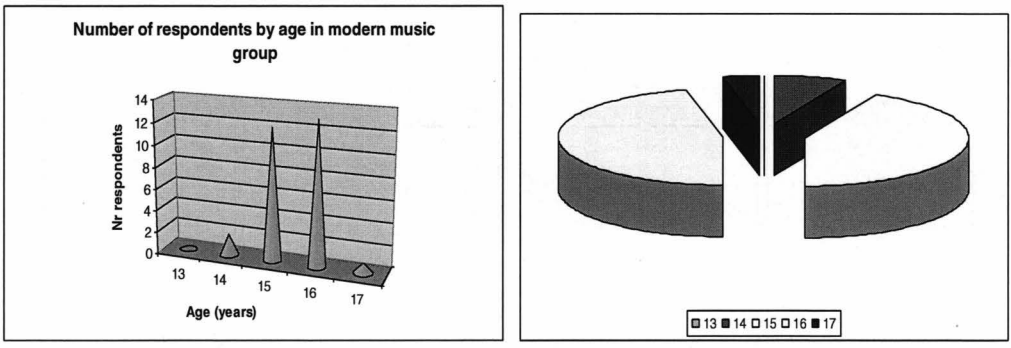

Fig. 4. Expression of respondent group by age in the presence of modern music (numerical - left; percentage - right)

Graphical representation by age in the case of control group (without music) is shown in Fig. 5. In this group numerically best represented age groups were those between 15 and 17 years old.

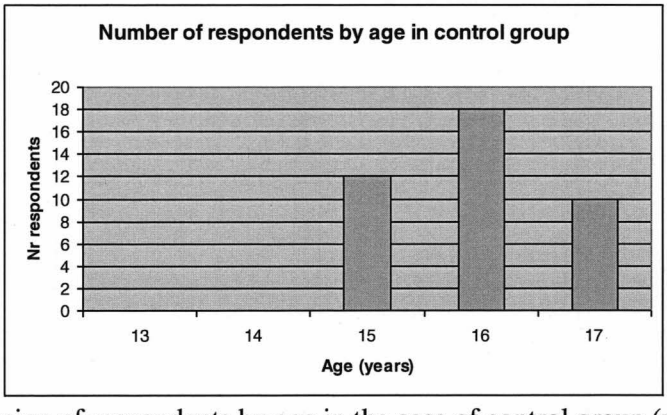

Fig. 5. Expression of respondents by age in the case of control group (without music)

In the study in which the subjects were asked if they prefer to learn or not in the presence of music one can see that 43.45% of the respondents prefer to learn in a quiet environment, without noise disrupters, and 56.54% of all respondents prefer to learn by listening to music in the background.

Analysis of the responses of the two tests is outlined below. Thus, in the first test (Fig. 6) based on the recall of as many objects in collage with 20 representations, in the case of the group exposed to hard-rock music one can observe that most of the respondents recalled from 45% to 50% of the images presented; the rest going from a minimum of 20% to 65%.

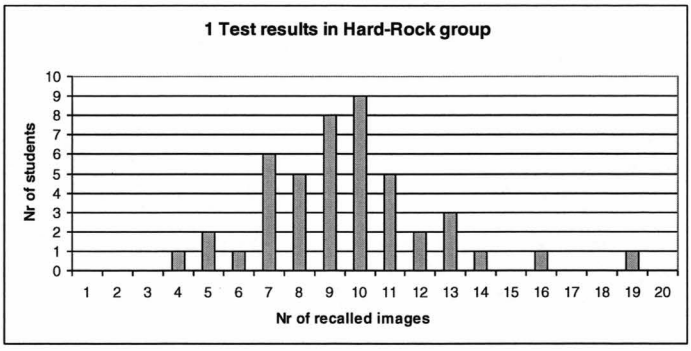

Fig. 6. First test results in the group exposed to hard-rock music

In the group exposed to classical music (Fig. 7) it is noticed that most of the subjects were able to recall 10 to 12 (50%-65%) of the items presented. In this situation we can see an increase of the images recalled between 7 and 13 (35%-65%) at most of subjects which better highlights their ability to concentrate compared to the group exposed to hard-rock music where the fewest records stood around 4.

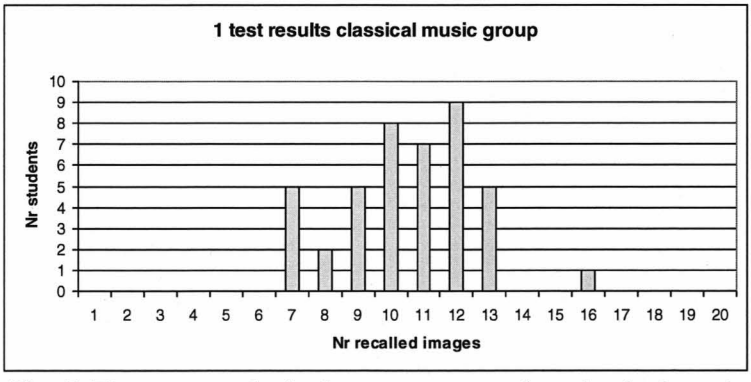

Fig. 7. First test results in the group exposed to classical music

In case of the group exposed to modern music (Fig. 8) one can see a broader sample of responses between 7 and 13 recalled images (35% -75%).

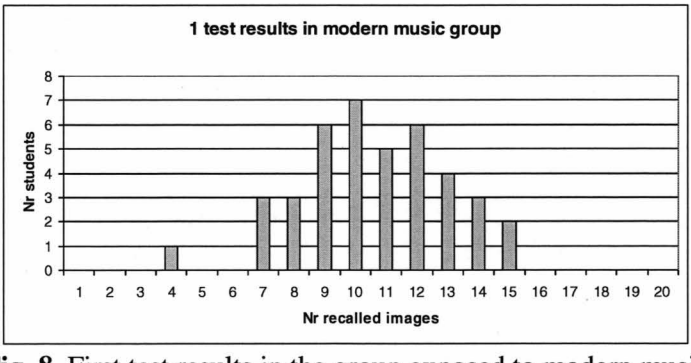

Fig. 8. First test results in the group exposed to modern music

In this situation, no one can say that the background noise was disturbing, one which adversely affect the ability to concentrate, but there is no memory stimulating factor.

In the control group which was kept under silence without any disturbing factor one can be seen better results regarding the possibility subjects to recall images shown (Fig. 9). They fail in bulk to recall between 60% and 90% of the pictures.

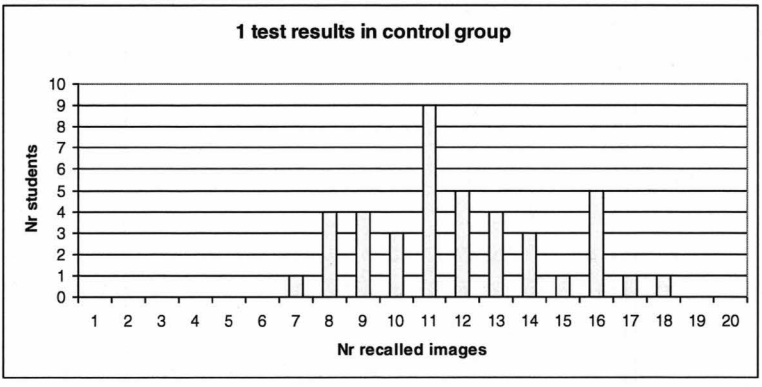

Fig. 9. First test results in control group

In the second test in which students had to recall a total of 12 words that were presented on the screen with a time of exhibition of 10 seconds per word, it can be seen that in the case of the group exposed to hard rock music (Fig. 10) the students were able to recall between 5 and 10 words. Most of them were able to recall 8 out of the 12 words presented or 60%.

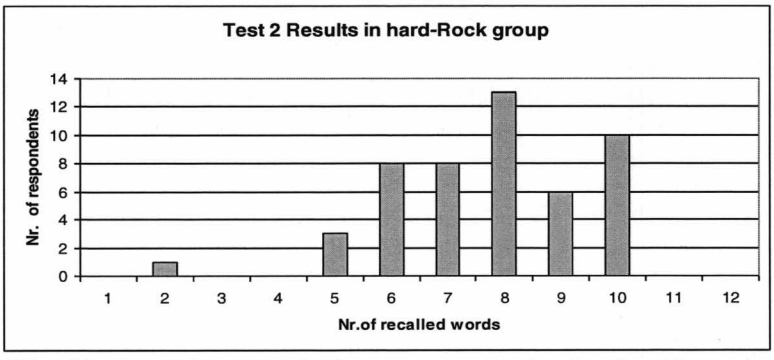

Fig. 10. Second test results in group exposed to hard-rock music

Noting the results reported for the group exposed to classical music (Fig. 11), an increase of words recalled is distinguished. Most subjects managed to recall from 6 to 12 words (50%-100%). Most have managed to reproduce 9 of 12 words presented or 75%. In this case we can clearly see an improvement in the students achievements.

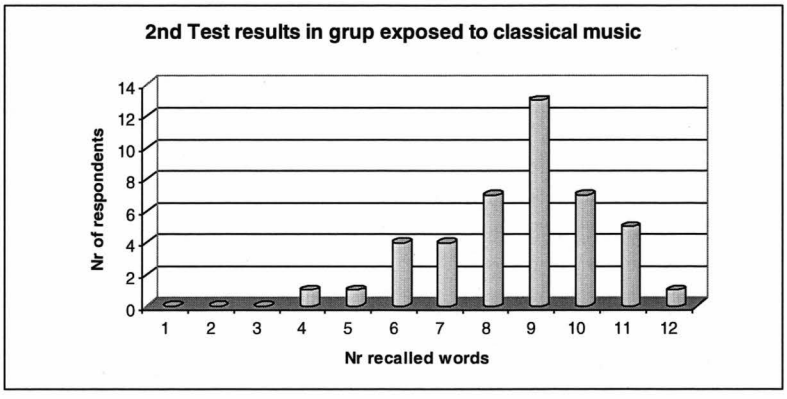

Fig. 11. Second test results in group exposed to classical music

The group exposed to modern music (Fig. 12) highlights much better results compared to the first two categories. In this case, one can observe a significant increase in the number of the words recalled. Most subjects fail to recall between 55% and 95% of the words presented. It can then be said that pop music is favored by most young people who are accustomed to this genre and more easily tolerate it. In other words, attention it is less distracted than in the other two situations.

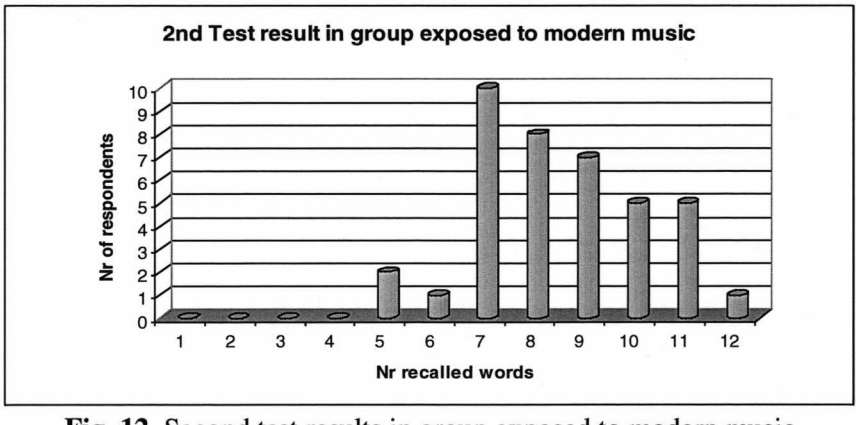

Fig. 12. Second test results in group exposed to modern music

Regarding the control group (Fig. 13) undergoing an experiment in silence, without music or any disturbing noise, the results show a significant increase in the number of the words recalled compared to other groups. In this group, most students were able to reproduce 60% of the words presented. Most were able to recall between 75% and even 100% of the words, which very clearly highlights the positive effect on concentration and memory capacity of quiet spaces or lack of any disturbing sounds of attention.

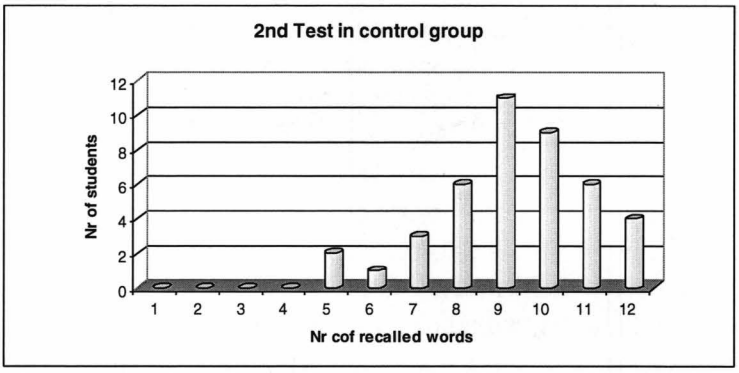

Fig. 13. Second test results in control group

Our study revealed for consignments tested in the presence of music regardless the applied musical type. A better performance achieved by the students who used to study in the presence of music, compared to those who prefer quiet spaces (Fig. 14). For those ones, background noise is not a disturbing negative factor, they tolerate noisy environments easier and their power of concentration is higher than in the case of subjects who prefer silence when studying.

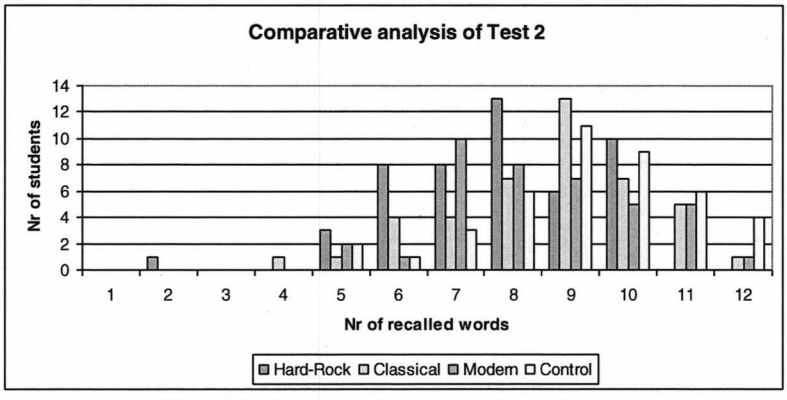

Fig. 14. Comparative analysis of Test 2

Also, one can see how students' intellectual performance is negatively affected by hard rock music, and increase in the presence of chamber music or pop music (Fig. 14).

Best results obtained with light music can be explained by the familiarity of the young people with this kind of music. Their attention being drawn by the sound in the background, as was the case with the music which is less familiar. For some students in our study classical music was a curiosity. There were students who asked about the composer, could easily render the area to which it belonged. This explains the reduction in the number of positive responses for classical music. The research results showed that there were

significant differences in the number of correct answers of the two groups (experimental and control), thus proving that the process of short memorizing can be disrupted by external factors such as music, sounds, noise, while in a peaceful atmosphere this process occurs normally.

Conclusions

This research, we have proposed it, clearly demonstrated that the process of short memorizing is hampered by disturbing factors, one of which in our case was music. It influenced more subjects' ability to remember certain details of the images that they had to look carefully.

This difference was observed when we used more an another group, the control group was subject for the experiment, which received much better results on memorizing items, compared to the experimental group.

Of course we can not forget that there are certain exceptions, these are people who memorize better in a noisy space, where there are many factors that could diminish memory and concentration. In this case, however most subjects have shown that these factors have ruffled more when they focus on exercise.

Sound, music, noise deters attention and ultimately affects the short memorizing process. Some subjects even expressed interest in the song they heard were listening to, so it was the object of attention of these subjects.

Noisy environments, classes with high numbers of students can be a negative factor in the learning process, such as memory capacity or retention of the information presented in the classroom, compared with grades with lower actual numeric students.

The increased number of information due to the overload curriculum can be a negative factor in terms of students' ability to retain information as accurately received during a typical day of school where the student has different courses of 6-7 hours a day and hourly rate he receives about 10 new informations (definitions, formulas, specialized scientific terms etc.).

We can say that students' school performance is influenced by the number of the class factor and the excessive number of information that they need to retain or understand.

Rezumat. Studiile au evidenţiat faptul că muzica poate influenţa memoria şi capacitatea de învăţare, dar există o serie de factori care pot influenţa rezultatele.

În studiile noastre am plecat de la "efectul Mozart" care susţine că ascultarea muzicii clasice poate creşte performanţa cognitivă.

Am dorit să cercetăm care este efectul zgomotului de fundal (în acest caz al muzicii) asupra memoriei de scurtă durată la adolescenţi, ţinând cont de faptul că în zilele noastre cei mai mulţi tineri afirmă faptul că studiază în timp ce ascultă muzică, sau dacă zgomotul poate influenţa în orice fel capacitatea de a reţine anumite informaţii.

În cadrul proiectului nostru a participat un număr de 160 elevi cu vârste cuprinse între 13 şi 17 ani. Elevii au fost aleşi în mod aleator şi au fost formate 4

grupuri: 1 grup de control şi 3 grupuri experimentale. Fiecare grup experimental a fost constituit din câte 40 de elevi. Fiecare grup de elevi din lotul experimental a fost expus unui gen muzical (muzică clasică, muzică hard-rock şi muzică uşoară), în timp ce grupul de control a fost supus testării în condiţii de linişte fără ca asupra acestora să acţioneze niciun factor perturbator.

Experimentul a constat în aplicarea a două teste care urmăresc capacitatea memoriei vizuale şi spaţiale de scurtă durată.

Studiul nostru a demonstrat clar că procesul memorării de scurtă durată este influenţat negativ de către factori perturbatori, unul dintre aceştia în cazul nostru a fost muzica. Aceasta a scăzut cu mult capacitatea subiectului de a memora anumite detalii legate de imaginile pe care trebuia să le privească cu multă atenţie, comparativ cu mediile liniştite în care acest proces are loc normal.

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ISSN 2069 - 1521