UDK 66:54(05)
CODEN HMIDA 8
ISSN 0367–598 X

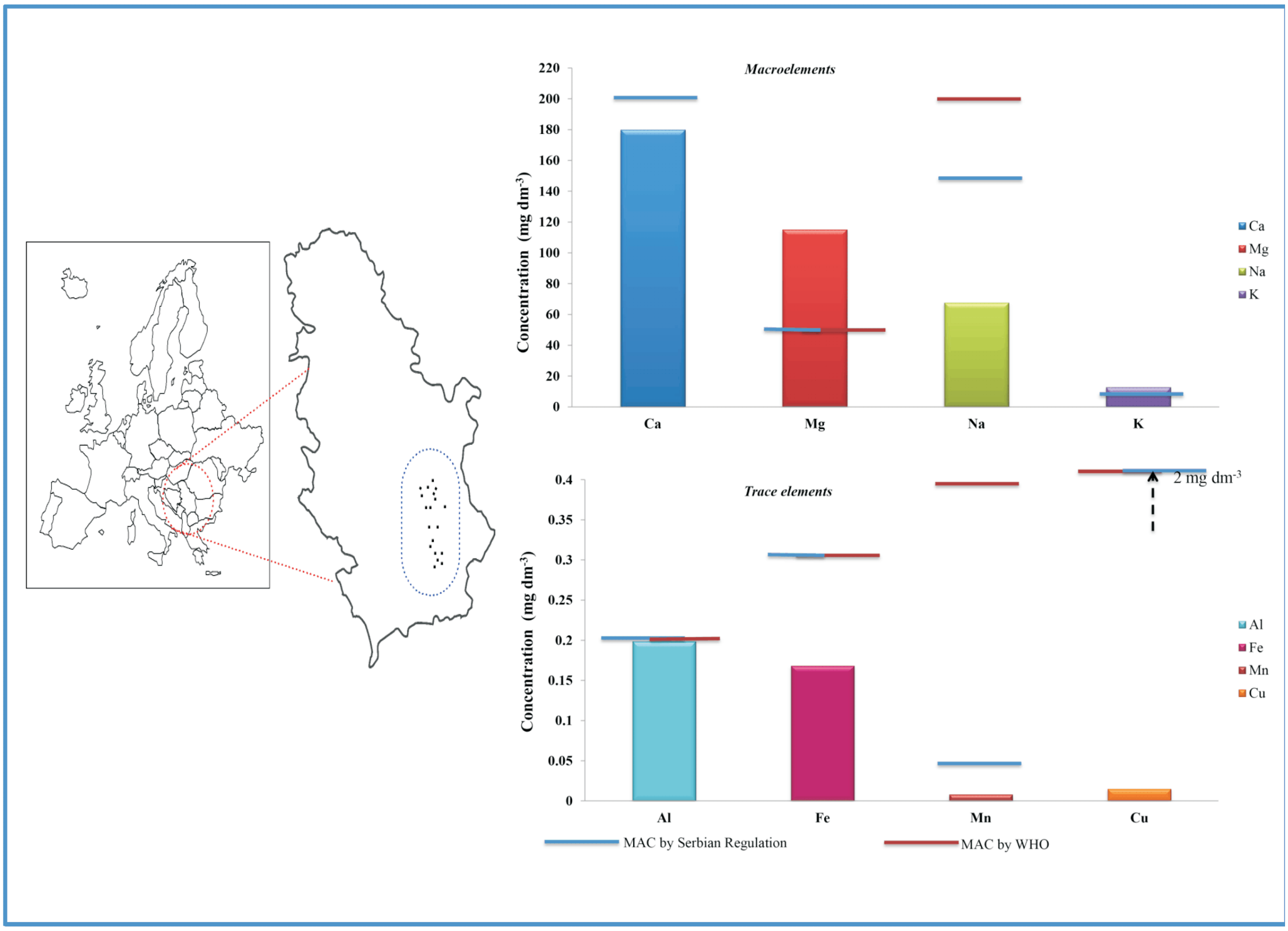
2

Vol. 69

Hemijska industrija

Časopis Saveza hemijskih inženjera

Chemical Industry



BEOGRAD, MART-APRIL 2015.
HMIDA 8, 69 (2) 107-217 (2015)

Chemical Industry
Химическая промышленность

Hemijska industrija

Časopis Saveza hemijskih inženjera Srbije
Journal of the Association of Chemical Engineers of Serbia
Журнал Союза химических инженеров Сербии

VOL. 69 **Beograd, mart–april 2015** **Broj 2**

Izdavač
Savez hemijskih inženjera Srbije
Beograd, Kneza Miloša 9/I

Redakcija
11000 Beograd, Kneza Miloša 9/I
Tel/fax: 011/3240-018
E-pošta: shi@yubc.net
www.ache.org.rs

Izlazi dvomesečno, rukopisi se ne vraćaju

Za izdavača
Tatijana Duduković

Sekretar redakcije
Slavica Desnica

Izdavanje časopisa pomaže
Republika Srbija, Ministarstvo prosvete, nauke i tehnološkog razvoja

Uplata pretplate i oglasnog prostora vrši se na tekući račun Saveza hemijskih inženjera Srbije, Beograd, broj 205-2172-71, Komercijalna banka a.d., Beograd

Kompjuterska priprema
Vladimir Panić

Štampa
Razvojno-istraživački centar grafičkog inženjerstva, Tehnološko-metalurški fakultet, Univerzitet u Beogradu, Karnegijeva 4, 11000 Beograd

Indeksiranje
Radovi koji se publikuju u časopisu *Hemijska Industrija* ideksiraju se preko *Thompson Reuters Scietific®* servisa *Science Citation Index - Expanded™* i *Journal Citation Report (JCR)*, kao i domaćeg *SCIndeks* servisa Centra za evaluaciju u obrazovanju i nauci

SADRŽAJ

CONTENTS

GENERALNI POKROVITELJ

HEMOFARM KONCERN

VRŠAC, Beogradski put bb, tel. 013/821-345, 821-027, 821-129
BEOGRAD, Prote Mateje 70, tel. 011/344-26-63, faks: 344-17-87
E-pošta: info@hemofarm.com

IZDAVANJE ČASOPISA POMOGLA JE:

INŽENJERSKA KOMORA SRBIJE
Bulevar vojvode Mišića 37
11000 Beograd

SUIZDAVAČI

Tehnološko-metalurški fakultet
Univerziteta u Beogradu, Beograd

Prirodno-matematički fakultet Univerziteta
u Novom Sadu, Novi Sad

Hemijski fakultet
Univerziteta u Beogradu
Beograd

Institut za tehnologiju nuklearnih i drugih
mineralnih sirovina, Beograd

HIP Petrohemija a.d. Pančevo

Tehnološki fakultet Univerziteta
u Novom Sadu, Novi Sad

Tehnološki fakultet Univerziteta
u Nišu, Leskovac

NU Institut za hemiju,
tehnologiju i metalurgiju
Univerziteta u Beogradu,
Beograd

„Nevena Color“ d.o.o.
Leskovac

DCP Hemigal, Leskovac

Ekološko i koroziono ponašanje osiromašenog uranijuma

Mirjana D. Stojanović[1], Časlav M. Lačnjevac[2], Marija L. Mihajlović[1], Marija V. Petrović[1], Tanja D. Šoštarić[1], Jelena T. Petrović[1], Zorica R. Lopičić[1]

[1]Institut za tehnologiju nuklearnih i drugih mineralnih sirovina, Beograd, Srbija
[2]Poljoprivredni fakultet, Univerzitet u Beogradu, Zemun, Srbija

Izvod

Poslednjih decenija se sve više govori o "*new pollutant*" – osiromašenom uranijumu (OU), koji se koristi u protivtenkovskim penetratorima zbog svoje velike gustine, prodornosti i pirofornih svojstava. Procenjuje se da je za vreme NATO agresije na bivšu Jugoslaviju 1999. god., približno 10 t osiromašenog uranijuma uneto u životnu sredinu, uglavnom na poljoprovredno zemljište. U vreme tih dešavanja je bilo vrlo malo dostupnih informacija o ponašanju ekoloških sistema narušenih ostacima penetratorima sa OU. Danas, nakon četrnaest godina, sve više se susrećemo sa "nevidljivom pretnjom" upotrebe municije sa OU, koji ima snažan radioaktivni i hemotoksični uticaj na ljudsko zdravlje. Ovaj rad pruža pregled korozionog i ekološkog ponašanja OU, zajedno sa indikatorima uticaja na životnu sredinu, sa ciljem da se istaknu oblasti koje zahtevaju dodatnu pažnju u izradi remedijacionih programa.

Ključne reči: osiromašeni uranijum, korozija, ponašanje, životna sredina.

Dostupno na Internetu sa adrese časopisa: http://www.ache.org.rs/HI/

PREGLEDNI RAD

UDK 504.5(497.1)"1999":
546.791623.454.8:631.4(497.1)

Hem. Ind. **69** (2) 107–119 (2015)

doi: 10.2298/HEMIND131025024S

URAN U ŽIVOTNOJ SREDINI - NORM/TENORM U SRBIJI

Radionuklidi u životnoj sredini mogu biti prirodnog, geološko-geohemijskog i antropogenog porekla. Antropogena radioaktivnost generisana je putem nuklearnih, vojnih i nenuklearnih (industrijskih) aktivnosti. Prirodna doza radioaktivnosti predstavlja približno 81% godišnje doze zračenja, dok preostalih 19% čini radioaktivnost poreklom iz antropogenih izvora [1]. Procenjuje se da je nuklearna katastrofa u Černobilu povećala prirodnu dozu zračenja za oko 2% [2]. Glavni doprinos prirodnoj radioaktivnosti je NORM – "*Naturally Occurring Radioactive Material*" i odnosi se na prirodne radioaktivne materijale koji se nalaze u životnoj sredini [3]. TENORM – "*Technologically-Enhanced Naturally-Occurring Radioactive Materials*", ili tehnološki povišen NORM, odnosi se na radioaktivne materijale čija je količina u životnoj sredini povećana ljudskim aktivnostima, odnosno, porasla iznad nivoa njihovog prirodnog stanja u životnoj sredini [4].

Prirodni izvori urana u Srbiji (NORM) su magmatske, sedimenentne, karbonatne stene i graniti, prosečnog sadržaja do 3.5 gU/t i predstavljaju „osnovu prirodnog nivoa jonizujućeg zračenja". Prirodno povećan sadržaj urana pronađen je u planinskim regionima, Bukulje, Janje, Stare Planine, Avale-Kosmaja, Rudnika, Cera, Vranja, Fruške Gore i Vršaćkog brda, u proseku od 50–200 Bq/kg [5]. Tehnološki procesi u elektranama, prerada fosfata, proizvodnja i primena fosfatnih đubriva, ostaci municije sa OU predstavljaju glavni oblik TENORMa [6]. Tako, u blizini termoelektrana „Kolubara" i „Nikola Tesla", jačina ekvivalentne doze zračenja varira od 1.,42–4,87 nSv/h, što je 3 do 4% iznad prirodnog nivoa zračenja [7]. Proizvodnja i upotreba fosfatnih đubriva značajno doprinosi povećanju sadržaja urana u životnoj sredini i globalnog je karaktera. Oko 73% antropogenog unosa urana u životnu sredinu, na svetskom nivou, poreklom je iz fosfatnih mineralnih djubriva. Sadržaj urana u fosfatnim rudama može da dostigne i do 300 g/t, u zavisnosti od porekla. Na osnovu svetske godišnje potrošnje od oko 135 miliona tona fosfatnih ruda, sa prosečnim sadržajem oko 150 gU/t, procenjuje se da se njihovom preradom može uneti oko 21.000 t urana u životnu sredinu. Na osnovu podataka da se u Srbiji godišnje na njive unese oko 1 milion tona veštaćkih đubriva na bazi fosfora, procenjuje se da se sa tim količinama unese u životnu sredinu vise od 200 t urana [8].

Napuštena ležišta i rudnici urana predstavljaju značajne generatore urana, tako jalovina zatvorenog rudnika urana u Gabrovnici–Kalna, sadrži od 15,33 do 17 mgU/kg. Voda koja izvire i danas iz rudnika, sadrži 0,053 mg U/dm^3 vode. Vegetacioni eksperimenti u realnim i kontrolisanim uslovima sa različitim vrstama biljaka (usevi i povrće), pokazali su da je sadržaj urana u biljkama uzgajanim na jalovini u Kalni znatno veći od sadržaja urana u biljkama uzgajanim na nekontaminiranom zemljištu, a stepen usvajanja urana znatno zavisi od vrste biljaka, genotipova i biljnih organa [1,9–11].

Prema nekim procenama „prirodan nivo jonizujućeg zračenja" u nekim sredinama Srbije je povećan 30 puta u poslednjih 30 godina, prouzrokovan ljudskim aktivnostima [7]. Nažalost, doprinos TENORMu u Srbiji je

---

Prepiska: M.D. Stojanović, Institut za tehnologiju nuklearnih i drugih mineralnih sirovina , Franše d'Eperea 86, Beograd 11 000, Srbija.
E-pošta: m.stojanovic@itnms.ac.rs

Rad primljen: 25. oktobar, 2013
Rad prihvaćen: 20. januar, 2014

bila i upotreba municije sa osiromašenim uranijumom za vreme NATO bombardovanja 1999. god., što je predmet daljeg izlaganja.

OSIROMAŠENI URANIJUM – KARAKTERISTIKE

Upotreba urana kao nuklearnog goriva, koji prirodno sadrži 0.7% ^{235}U, zahteva njegovo obogaćivanje ovim izotopom do sadržaja od 3 do 5%. U nuklearnom gorivnom ciklusu generiše se osiromašeni uranijum kao sporedni proizvod sa sadržajem izotopa ^{235}U od 0,2–‍–0,3%. Tako dobijeni osiromašeni ^{238}U nije više ekonomski isplativ i ima status radioaktivnog otpada. Specifična aktivnost OU je oko 14,83 Bq/mg, i za 30–40% je manje radioaktivan od prirodnog uranijuma (25,4 Bq/mg), sa istim poluvremenom raspada od 4,468×10^{9} godina [12].

Procenjuje se da se širom sveta približno 1,1 milion tona OU nalazi na raznim deponijama, pri čemu se na ovu količinu svake godine doda još minimum 46 hiljada tona [13].

Upotreba municije sa DU u prethodnim ratovima

Tokom svih ratova u prethodnih 20 godina (Zalivski rat 1991, rat u Bosni 1994, bombardovanje Srbije 1999. i invazija na Irak 2003) ispaljeno je približno 1,4 miliona projektila sa OU. Tabela 1 prikazuje podatke o unosu OU u životnu sredinu, prikupljenih iz različitih izvora na osnovu kojih se može zaključiti da ne postoje precizni podaci o tačnim količinama upotrebljene municije [14–‍–21].

Fizičko ponašanje metka sa osiromašenim uranijumom nakon ispaljivanja

Osiromašeni uranijum je odobren od strane Ministarstva odbrane SAD za izradu municije i projektila koji sadrže 0,2% izotopa ^{235}U i 0,0003% izotopa ^{236}U. Penetrator (udarna igla) napravljen je od legure koja se sastoji od OU (99,25%) i titana (0,75%). Osiromašeni uranijum odlikuje velika gustina 19,05 g/cm^{3}, što je 70% više od olova (11,35 g/cm^{3}), niska tačka topljenja (1.132 °C), piromorfnost i velika probojna moć (kalibrom od 30 mm može da se probije čelik debljine 6–9 cm) [13].

Prilikom ispaljivanja municije prosečno 10–35% (maksimalno 70%) od mase penetratora se pretvara u aerosol [14]. Znatno manje aerosola se proizvodi u slučaju kada penetrator promaši cilj, pri čemu se oko 90% deponuje u zemljištu [22]. Prilikom udara u metu, 18–70% tela penetratora se zapali i oksiduje, a fragmenti se talože (deponuju) po površini zemljišta u vidu finih čestica ili sagorevanjem prelazi u okside uranijuma u vidu aerosola [13]. Zbog svoje termohemijske nestabilnosti, primarno nastaje nestehiometrijski U(VI) oksid oblika UO_{2+x} (gde je $0<x<0,4$), dok pri daljoj oksidaciji dolazi do obrazovanja smeše oksida U(IV) i U(VI). Od nastalih oksida uranijuma, UO_2 nije rastvoran u vodi, a UO_3 je rastvoran i gradi uranil jon, UO_2^{2+}, lako pokretljiv u životnoj sredini [13].

Kretanje projektila sa osiromašenim uranijumom nakon udara u čvrstu metu doprinosi kontaminaciji životne sredine putem [13]:

– Dubinske kontaminacije zemljišta usled penetracije neoštećenih (nesagorelih) projektila, U neposrednoj okolini pogođenog mesta kontaminacija je najveća i uglavnom projektili korodiraju u hidratisani U(VI) oksid, veoma rastvornog u vodi [23].

– Tačkaste kontaminacije: kontakt sa ostacima sagorelih projektila. U slučaju da ostaci sagorelih projektila ostanu na površini zemljišta može doći do tačkaste kontaminacije, neposredno oko mesta pada. Na ovim lokacijama tlo je površinski kontaminirano sitnim fragmentima i oksidima OU ili delovima košuljice projektila. Na rtu Arza, u Crnoj Gori, konstatovano je 144 ovakvih lokacija [24].

– Površinske kontaminacije: nastanak aerosola sa oksidima uranijuma. Prilikom udara penetratora sa OU u čvrstu metu i nastanka plamena, oksidi uranijuma grade aerosole, koji se sastoje od čestica mikroskopske veličine. Po nekim podacima iz literature, 50–96% čestica u vazduhu su prečnika manjeg od 10 µm (uglavnom oko 5 µm), a 17–48% ovih čestica je rastvorno u vodi [13].

Tabela 1. Količine OU (t) dispergovane u životnu sredinu tokom prethodnih ratova
Table 1. Amount of DU (t) is dispersed into the environment during previous wars

Zemlja	Period konflikta	OU dispergovan u životnu sredinu, t	Izvor
Irak i Kuvajt Zalivski rat	1990–1991	300–321	[14,15,16]
Zalivski rat	1990–1991	258	[14]
Bosna–Hercegovina	1994–1995	2,75	[14]
Bosna–Hercegovina	1995	3	[14,15]
Srbija i Crna Gora (konflikt na Kosovu)	1999	10	[14,17,18,19]
Srbija i Crna Gora (konflikt na Kosovu)	1999	11	[14,15]
Konflikt na Kosovu	1999	8,5	[14]
Rat u Iraku	2003	170–1700	[20]
Rat u Iraku	2003	>30	[21]

KOROZIONO PONAŠANJE OSIROMAŠENOG URANIJUMA

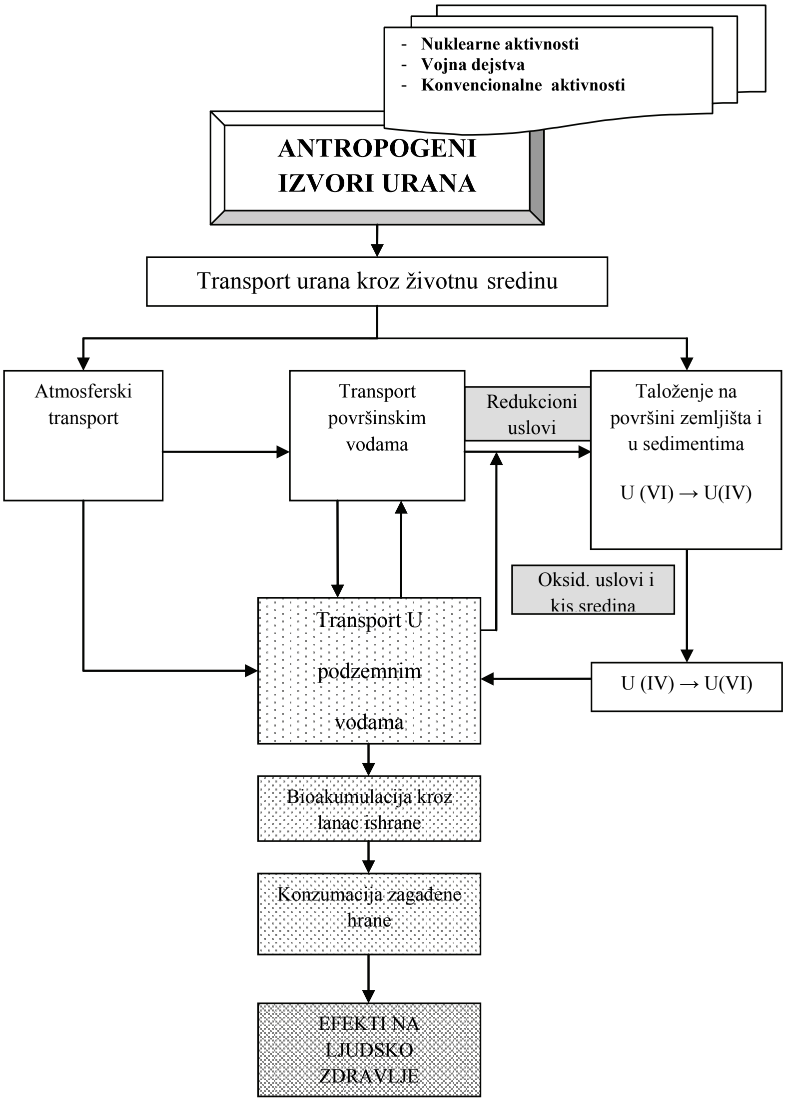
Migracioni potencijal urana zavisi od fizičko-hemijskih osobina zemljišta i zemljišnih rastvora i oksidacionih proizvoda OU. Na mobilnost rastvorenih proizvoda urana, dominantno utiču pH, *Eh*, i prisustvo kompleksirajućih organskih i neorganskih agenasa u lokalnim podzemnim vodama i zemljištu.

Šestovalentni uran U(VI), postoji u rastvoru kao uranil jon (UO_2^{2+}) i on je mobilniji od U(IV) jer lakše gradi rastvorne komplekse sa ligandima prisutnim u zemljišnim rastvorima. Pokazalo se da je prisustvo karbonata i fosfata, takođe utiče na ove procese. Transport rastvornog oblika urana, može biti ubrzan razblaživanjem, pošto se time smanjuje njegova koncentracija u podzemnim i površinskim vodama. Ove reakcije uključuju jonsku izmenu i specifičnu adsorpciju urana organskim supstancama, mineralnim glinama, Fe(III) i hidroksida prisutnih u zemljištu.

Ciklus kretanja urana (identično ponašanje ima i OU) u životnoj sredini kao i potencijalni rizik na ljudsko zdravlje, prikazani su na slici 1.

Kako će se u tim slučajevima penetrator ponašati i do koje dubine će prodirati zavisi od ugla ulaska u tlo i fizičkih osobina zemljišta. Tako, na glinovitim zemljištima, prodor ide do 2 m [22], a na peskovitim zemljištima od 6–7 m [17].

Studije sprovedene od strane UNEP-a, u postkonfliktnim zonama, su otkrile brojne korodirane penetratore u površinskim slojevima zemljišta [17,25,26]. U Srbiji i Crnoj Gori su tokom 2002. god., sa površine zemljišta, uzorkovani penetratori visokog stepena korozije i procenjuje se da bi po utvrdjenoj stopi korozije



Slika 1. Ciklus kretanja urana [50] (Slika je preuzeta uz ljubaznu saglasnost izdavača monografije ITNMS, Beograd).
Figure 1. Cycle of uranium movement [50] (The figure was taken with the kind permission of the publisher monograph ITNMS, Belgrade).

penetrator potpuno korodirao za 20 godina [27]. Nakon 18 meseci od agresije, nađen je penetrator u Đakovici na 5 cm dubine, sa gubitkom mase od 2–8%, usled oksidacije i spiranja atmosferskim padavinama, a ostaci OU nadjeni su ispod penetratora na dubini od 12,5 cm. Na osnovu iznetog, procenjeno je da se za 15–30 godina, penetrator može potpuno rastvoriti (pređe u jedinjenje $UO_3 \cdot nH_2O$) [28].

Sakupljeni uzorci na na Kosovu su takođe pružili uvid o veličini, sastavu i distribuciji čestica OU, nastalih tokom abrazije, sagorevanja ili korozije penetratora. Autori su izvestili da su hiljade čestica OU bile prisutne u zemljištu uzorkovanog sa 0 do 15 cm dubine, ispod jednog penetratora [29]. Analizom skenirajućim elektronskim mikroskopom X-zraka (SEM-XRMA) utvrdjeno je da je prosečna veličina čestica manja od 2 μm čija dalja karakterizacija je pokazala da je 50% svih čestica sastavljeno od UO_2, sa preostalim česticama sačinjenim od U_3O_8 ili mešavine ovih oksidacionih oblika. Na uzorcima zemljišta sa Kosova, potvrdjeno je da su čestice OU veličine ispod 1 μm, pretežnog sastava visoko nerastvornog UO_2, s tim, da svaka analizirana čestica sadrži mali deo mobile U(VI) faze [30].

Slična veličina čestica OU, potvrđena je i na uzorcima zemljišta prikupljenih u Kuvajtu od strane IAEA tokom UNEPovih postkonfliktnih terenskih istraživanja [31]. Ispitivanja aerosola i čestica OU u unutrašnjem i spoljašnjem delu borbenih vozila nakon dejstva penetratora, pokazala su heterogenost u morfologiji čestica a XRD analiza mešavinu oksida urana – U_3O_8/UO_3 i U_4O_9 sa prisustvom manjih količina šepiotita, $UO_3 \cdot 2H_2O$ [32].

Osiromašeni uranijum je termodinamički nestabilan i stoga je očekivana njegova korozija u prirodnim sistemima. Korozija i rastvorljivost OU se javlja u dve faze [33,34]:

1) oksidacija metalnog uranijuma, nulto valentnog stanja, do U(IV), koji u prirodi gradi mineral, uraninit - UO_2. (jednačine (1) i (2)),

2) oksidacija U (IV) do U (VI) (jednačine (3) i (4)).

Prva faza je povoljnija sa stanovišta zaštite životne sredine jer nastaju nerastvorni produkti koji pod određenim zemljišnim uslovima (pH i redoks potencijal) prelaze u fazu 2, koja dovodi do nastajanja rastvorljivih vrsta, kao što su uranil joni (UO_2^{2+}) (jednačina (3)) i minerala, npr., šepiotia ($UO_3 \cdot 2H_2O$) (jednačina (4)), a koji pod određenim uslovima može da otpusti UO_2^{2+} jone, koji se lako transportuju zemljišnim rastvorima i uključuju u lanac ishrane (jednačina (5)):

$$U_{(s)} + 2H_2O \rightarrow UO_{2(s)} + 2H_2 \quad (1)$$

$$U_{(s)} + O_2 \rightarrow UO_{2(s)} \quad (2)$$

$$UO_{2(s)} + 2H^+ + 0{,}5O_2 \rightarrow UO_2{}^{2+}{}_{(aq)} + H_2O \quad (3)$$

$$UO_{2(s)} + 0{,}5O_2 + 2H_2O \rightarrow UO_3 \cdot 2H_2O_{(s)} \quad (4)$$

$$UO_3 \cdot 2H_2O_{(s)} + 2H_2{}^+ \rightarrow UO_{2(aq)}{}^{2+} + 3H_2O \quad (5)$$

U zavisnosti od redoks potencijala i pH, metal uranijuma može u vodi da korodira, da se pasivizira ili da bude inertan. U domenu korozije dolazi do degradacije metala, dok u domenu pasivizacije, metal postaje obložen oksidima, hidroksidima i hidridima ili solima, dajući različite stepene zaštite od dalje korozije, a u domenu inertnosti korozija metala nije termodinamički moguća [35]. Proces korozije OU–Ti legure je nižeg intenziteta u odnosu na prirodni uran [36–38].

Sa druge strane, uran se u prirodi oksiduje zbog priliva kiseonika, odnosno porasta njegove fugasnosti koji dolazi sa površinskim vodama, descedentno niz pukotine. Ovi rastvori su ujedno i blago kiseli, jer sadrže CO_2, koji sa vodom gradi ugljenu kiselinu. Afinitet prema kiseoniku je takav, da će se prvo oksidovati sulfid do sulfata, U(IV) do U(VI), a tek pri višem redoks potencijalu, Fe^{2+} do Fe^{3+}, prema reakcijama (6)–(8):

$$H_2S + 4H_2O = SO_4{}^{2-} + 10H^+ + 8e^-,\ E^0 = 0{,}303\ V \quad (6)$$

$$U^{+4} + 2H_2O = UO_2{}^{2+} + 4H^+ + 2e^-,\ E^0 = 0{,}327\ V \quad (7)$$

$$Fe^{2+} + 3H_2O = Fe(OH)_3 + 3H^+ + e^-,\ E^0 = 1.06\ V \quad (8)$$

Proces taloženja urana redukcijom od neprocenjivog je značaja, jer isključuje uran iz vodenih tokova i samim tim obustavlja njegov proces širenja kroz životnu sredinu. Redukcija mobilnog jona urana (U^{6+}) do nerastvornog oblika urana (U^{4+}) odvija se onda kada opadne fugasnost kiseonika u rastvoru, tako da se ova reakcija odvija na teret oksidacije gvožđa ili sumpora. Ukoliko Fe^{2+} ima više u odnosu na kiseonik, istrošiće se kiseonik i tada kao oksidaciono sredstvo služi uranil jon koji prevodi Fe^{2+} do Fe^{3+}, ili sulfid do sulfata, i sam se pri tom taloži, što je prikazano reakcijama (9–(11) [39]:

$$UO_2 + 2Fe^{2+} + 3H_2O = UO_2{}^0 + 2\ Fe_2O_3 + 6H^+ \quad (9)$$

$$2Fe^{2+} + 0{,}5O_2 + H_2O = FeO\ (OH) + H^+ \quad (10)$$

$$4UO_2{}^{2+} + S^{2-} + 4H_2O = 2UO_2{}^0 + SO_4{}^{2-} + 8H^+ \quad (11)$$

Ispitivanje mehanizma korozije OU u različitim uslovima, obuhvataju *in situ* i laboratorijska istraživanja, koja objedinjena daju doprinos razumevanju ove oblasti. U tabeli 2 dat je pregled svetskih studija u ovoj olasti, a tabela 3 pruža informacije o strukturi produkata korozije, što omogućava definisanje konceptualnog modela produkata korozije penetratora od osiromašenog uranijuma u različitim realnim uslovima (Slika 2).

Stopa korozije OU se može izračunati pomoću jednačine (12), koja podrazumeva linearnu koroziju OU. Nedavna istraživanja su utvrdila da nesagoreli penetrator OU prati latentni, korozioni period izmedju 33 i 242 dana, u zavisnosti od geohemijskih uslova [36,37], te se preporučuje da se ovaj latentni period oduzme od ukupnog vremena čime će se dobiti tačnija stopa korozije na godišnjem nivou [16]:

Tabela 2. Stopa korozije OU–Ti legure u različitim uslovima životne sredine
Table 2. The rate of corrosion of DU–Ti alloys in different environmental conditions

Br.	Uslovi životne sredine	Geohemijski uslovi	Stopa korozije g cm^{-2} god^{-1}	Dodatni uslovi	Referenca
1	Vazduh	Oksidacioni	0,0012	Laboratorijski vazduh (30 dana)	[40]
2	H_2O	Oksidacioni	0,072	Destilovana voda	[40]
3	Rastvor 3,5% NaCl	Oksidacioni	0,40	–	[40]
4	5% NaCl	Oksidacioni	1,5	–	[41]
5	Morska voda, Solway Firth	Oksidacioni	2,6–3,1	*In situ*	[38,42]
6	Marina sediment, Solway Firth	Oksidacioni	1,4–1,8	*In situ*	[38]
7	Marina sediment u lab,uslovima	Radukcioni	0,056±0,006	Salinitet 31,5; pH 7,6–7,9, 0,8% org. mat., *CEC* 1,3 meq/100 g	[36]
8	Vlažan pesak (lab. uslovi)	Ultra redukcioni	0,02±0,003	Salinitet 31,5; pH 7,6–7,9; 0,8% org. mat.; *CEC* 1,3 meq/100 g	[36]
9	Pesak, Eskmeals Cumbia	Nedefinisani	0,08–0,17	pH 6,5–7,9	[38]
10	Pesak, Eskmeals Cumbia	Oksidacioni	0,10±0,01	pH 7,2–7,5; 0,8% org. mat.; *CEC* 1,3 meq/100 g	[37]
11.	Orgasnko zemljište bogato glinom	Nedefinisani	0,8–1,1	pH 5,8–6,0	[38]
12.	Vlažna zemljišta	Oksidacioni	0,49±0,06	pH 5,0–6,5, 12% org. mat.; *CEC* 21 meq/100 g; vlaga 22%	[37]
13.	Plavno zemljište	Redukcioni	0,010–0,02	pH 5,0–6,5; *CEC* 21 meq/100 g	[37,47]
14.	Zemljišta đubrena fosfotnim đubrivom	Redukcioni	0,00016–0,0044	27–45 P_2O_5 mg kg^{-1};pH 5,0–6,0; 12% org. mat.; *CEC* 21 meq/100 g	[37]

$$\text{Stopa korozije (g cm}^{-2}\text{ god}^{-1}) = \frac{365\times\text{Gubitak mase(g)}}{\text{Površina metala (cm}^{2})\times\text{Vreme (dani)}} \qquad (12)$$

Korozija OU u vodi

Proces korozije OU je ubrzana u vodi u odnosu na vazduh [40]. U laboratorijskim ispitivanjima je potvrđeno da prisusutvo hloridnih jona ubrzava proces korozije. Stopa korozije raste sa povećanjem sadržaja hlorida. Koroziona stopa iznosila je 0,07 g cm^{-2} god^{-1} u vodi , 0,40 g cm^{-2} god^{-1} u 3,5% rastvoru NaCl [40], a koroziona stopa od 1,47 g cm^{-2} god^{-1} u 5% rastvoru NaCl [41].

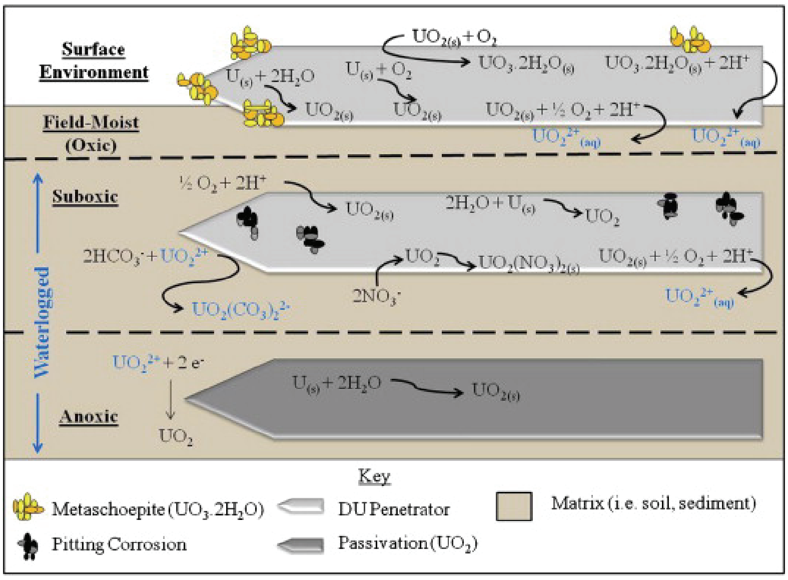
Sudbina nesagorelih penetratora od OU-Ti legure je ispitivana u slanim vodama Solvay Firth *in situ* i tom prilikom je utvrđena stopa korozije od 2,9 g cm^{-2} god^{-1}, koja je za red veličine veća od laboratorijskih rezultata koje je sproveo Trzaskoma i sar. [40] u približno istom sadržaju hloridnih jona (3,5% NaCl). Rezultat se pripisuje fizičkim procesima, kao što je abrazija od nanosa čestica pod turbulentnim strujanjima i klimatskim uslovima. Na površini penetratora je detektovano većinsko prisustvo uranitita UO_2 a u okolnim vodama prisustvo UO_2^{2+}.

Korozija OU u sedimentima, pesku i zemljištu

Sudbina nesagorelih penetratora od OU–Ti legure je takođe ispitivana *in situ* u površinskim sedimentima

Tabela 3. Produkti korozije OU pod različitim uslovima
Table 3. DU corrosion products under different conditions

Mineral – produkt korozije OU	Formula, produkti korozije OU	Uslovi	Izvor
Uranit	UO_2	Visoka vlažnost i nizak sadržaj kiseonika	[37,38,42]
Šoepit	$UO_3\cdot 2H_2O$	Niska vlažnost i dominantni oksidacioni uslovi	[37,38,42]
Metašoepit	$(UO_2)_8O(OH)_{12}\cdot 10H_2O$		
Uranfit	$NH_4(UO_2)\ (PO_4)\cdot 3H_2O$	Zemljište bogato fosfornim jonima	[42–44]
Hermikovit	$(H_3O)_2(UO_2)_2(PO_4)\cdot 6H_2O$		
Meta-autunit	$Ca(UO_3)\ (PO_4)\cdot 4H_2O$		
Sabugalit	$HA\ (UO_2)_4(PO_4)_4\cdot 16H_2O$		



Slika 2. Konceptualni model glavnih mehanizama korozije penetratora OU u zemljišnim uslovima [16] (Slika je preuzeta iz casopisa Sci. Total Environ. uz ljubaznu saglasnost ELSEVIER provided by Copyright Clearance Center).
Figure 2. Conceptual model of the major mechanisms of DU penetrators corrosion in soil conditions [16] (The figure is taken from the journal Sci. Total Environ. with the kind permission of ELSEVIER provided by Copyright Clearance Center).

od strane Solvay i Firth [38]. Koroziona stopa od 1,6 g cm^{-2} god^{-1} bila je znatno niža od one određene *in situ* u morskoj vodi. Sa druge strane, stopa korozije je bila veća od stope korozije određene u laboratorijskim uslovima u slanoj vodi definisanog sadržaja NaCl (Tabela 2; br. 3.) što ukazuje da su fizički procesi i klimatski uslovi imali značajan uticaj na proces korozije. Potvrda ove tvrdnje sprovedena je laboratorijskim ispitivanjima korozione stope sa istim sedimentima, u koloni sa morskom vodom, koja je iznosila 0.056 g cm^{-2} god^{-1} ali je bila mnogo sporija od one ustanovljene *in situ* na pesku pod istim uslovima (Tabela 2; br. 8.) [37].

Ispitivanje kinetičkog mehanizma korozije OU, bila su predmet ispitivanja *in situ*, u pesku u Eskmeals, Cumbria [38] i simuliranim eksperimentima [42]. Obe studije daju uporedive korozione stope između 0.080 i 0.17 i 0,10 g cm^{-2} god^{-1}, redom, sa sličnim strukturama korozionih naslaga [38]. Crni i žuti proizvodi korozije su detektovani kao UO_2, i šoepit [38] ili metašoepit [42]. Korozija prati linearni trend od 9% gubitka mase za 500 dana. Rezultati su značajni sa aspekta procene korzije penetratora u pustinjama Iraka i Kuvajta, s obzirom na nedostatak podataka o koroziji osiromasenog uranijuma u ovim sredinama. U alkalnim zemljištima šoepit i metašoepit su cementirani silicijumom, što limitira njihovu mobilnost.

Korozija OU ispitivana je *in situ* u glinovitim zemljištima Kirkcudbright, Škotska [37]. Mehanizam korozije je uporediv sa onim u pesku, sa crnim i žutim korozionim proizvodima sa dominantnim mineralima urana, UO_2, uranitit i $UO_3 \times 2H_2O$, šoepit, 102. dana nakon ispaljivanja i deponovanja u zemljištu. Međutim, izračunata stopa korozije je za red veličine veća nego u pesku Eskmeals i iznosi 0,80–1,1 g cm^{-2} god^{-1}.

Sličan mehanizam korozije je primećen kod penetratora, na bazi OU–Ti legure, prikupljenih sa dva tipa zemljišta sa Kosova (peščane i sedimentne ilovače). Uzorci su tretirani u laboratoriji na 20 °C sa veštačkom kišom [45]. Utvrđeni su produkti korozije crne i žute boje. Utvrđena je zanemarljiva razlika u korozionim stopama između ova dva tipa zemljišta (0,19±0,03 g cm^{-2} y^{-1}) koje su imale slične pH vrednosti (5,6–5,8) i isti sadržaj organskih materija (2,1%). Studija je takođe pokazala da korozija OU nije linearna i da se sa vre–menom ubrzava. U prvoj godini je korodiralo ~1,6% od OU a posle 3. godine, stopa korozije OU je povećana na 2,7% od OU, po godini [45].

Dokazano je da korozija OU veoma zavisi od sadržaja vlage u zemljištima. Pod zemljišno vlažnim uslovima, OU korodirala po stopi od 0,49±0,06 g cm^{-2} god^{-1}, a dominantni input urana u zemljištima je u obrazovanju minerala metašopita, $(UO_2)_8O_2(OH)_{12}\cdot 10(H_2O)$. Međutim, pod uslovima vodoplavnih zemljišta, stopa korozije je značajno niža i kretala se od 0.01–0.02 g cm^{-2} god^{-1}, a mehanizam korozije je uporediv sa drugim vodoplavnim medijuma kao što je pesak i drugi sedimenti (Tabela 2, br 7. i 8.), sa formiranjem UO_2 jedinjenja na površini penetratora, što je praćeno generisanje mobilnih UO_2^{2+} u zemljišno okruženje. Dakle, mehanizam korozije, pod vlažnim uslovima, obuhvata oksidaciju OU do metašopita i izdvajanja uranil jona pod oksidacionim uslovima u skladu sa jednačinama (1)–(5) [36].

Hendley-Sidhu i sar. postavili su jednačinu za izračunavanje ukupnog vremena potrebnog za potpunu koroziju "Charm 3" penetratora, približne mase 4500 g i 150 cm^2 površine (jednačina (13)) [37]:

$$\text{Potpuna korozija (godina)} = \\ = \frac{4500\ \text{g}}{\text{Stopa korozije (g cm}^{-2}\ \text{god}^{-1})\times 150\ \text{cm}^2} \qquad (13)$$

Na osnovu studije Toque i Bejker [38] procenjuje se da potpuna korozija nastupa posle 27 godina, prema Handlei-Sidhu [36] posle 61 godine i 20 godina prema UNEP [25].

Handley-Sidhu i sar. su sproveli studiju ispitivanja uticaja PO_4^{3-}, poreklom iz poljoprivrednih zemljišta, tretirana sa fosfatnim mineralnim djubrivima, na koroziju penetratora OU. Zemljišta su imala prosečan sadržaj P_2O_5 od 27 mg kg^{-1}. Stope korozije su ~7 puta manje, nego u uporedivim plavljenim zemljištima (tabela 2; br. 13. i 14.) na 0.00016–0.0044 g cm^{-2} god^{-1}) [16]. Ova stopa korozije je znatno niža od utvrdjenih u vazduhu ili vodi (tabela 2, br. 1. i 2.). Može se zaključiti da prisustvo PO_4^{3-} u zemljištu štiti od dalje korozije osiromašeni uranijum. Formiranje zaštitnog autunitnog i meta-autunitnih slojeva na površini OU potvrđeno je i laserskom indukovanom fluorescentnom spektroskopijom [44].

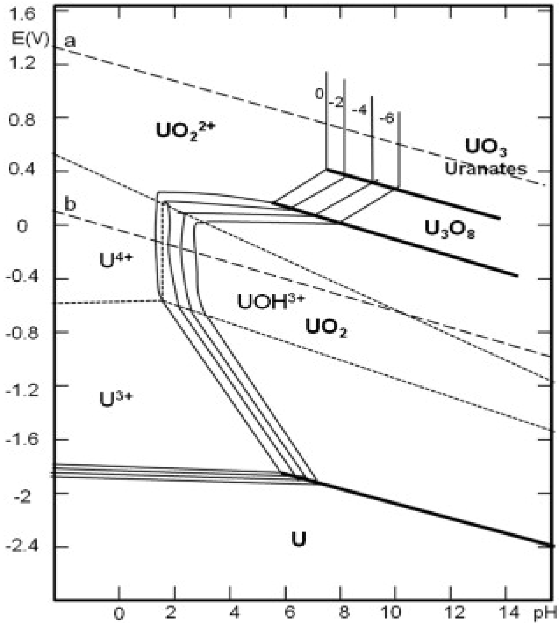
Uticaj biogeohemijskih uslova na koroziju osiromašenog uranijuma

Dosad je konstatovano da sadržaj vlage u zemljištima ili sedimentima, kontroliše mehanizam korozije OU. Izuzetno je važno utvrditi kako parametri zemljišta ili sedimenata, kao što su pH, kapacitet katjonske izmene (*CEC*) i sadržaj organske materije utiču na stopu korozije OU [46].

Veća stopa korozije na sedimenatima, u odnosu na pesak Solvej Fert (Tabela 1, 7 i 8), uprkos sličnm pH vrednostima, pripisuje se većem *CEC* (4,0 naspram 1,3 meq/100 g) i većem sadržaju organskog ugljenika (OC) (3,2% naspram 0,80%), tako da se i ovi faktori moraju uzeti u obzir pri proceni stope korozije [36].

Koroziona stopa je bila pet puta veća u vlažnim zemljištima (tabela 2, br. 12.) nego u vlažnom pesku na terenu (tabela 2, br 10.) [37,47], pri čemu su svi faktori korozivnosti veći u zemljištu. Zemljište je bilo više kiselo (pH 5,5 naspram 7,4) i imalo je veći sadržaj vlage (22% u poređenju sa 13 %), veći *CEC* (21 naspram 1,3 meq/100 g) i veći OC sadržaja (12% naspram 0,80%) u odnosu na pesak.

Kritičnu ulogu u kontroli korozije OU igra redoks potencijal, i odvija se pod oksidacionim uslovima, pri višku kiseonika i u skladu sa termodinamičkim uslovima i *Eh*/pH (Slika 3). Istovremena mobilizacija i imobilizacija, odnosno korozija OU i njegovo rastvaranje u oksidacionim uslovima je moguća, odnosno odigrava se oksidacija uranitita UO_2, u mobilne U(VI) jone [48]. Korozija OU prestaje pod redukcionim uslovima (prisustvo sulfata i gvožđe(II)) u različitim medijima (pesak, zemljište i zalivski sedimentni) uz formiranje uranitita (UO_2) i potpune pasivizacije metala [36,37].



Slika 3. Eh–pH dijagram stabilnosti uranijuma. Linije (a) i (b) odredjuju granice termodinamičke stabilnosti vode na 298 K, 1 atm. pritiska. Iznad linije (a), oksidacioni uslovi, a ispod linije (b), redukcioni uslovi. Isprekidane linije razgraničavaju jonske vrste i predstavljaju stanje u kome je termodinamička aktivnost jonskih vrsta sa obe strane linije ista [49].
Figure 3. Eh–pH stability diagram of uranium. Lines (a) and (b) sets limits on the thermodynamic stability of water at 298 K, 1 atm pressure. Above the line (a), oxidizing conditions and below the line (b) reducing conditions. Dashed lines delimit the ionic species and represent the state in which the thermodynamic activity of ionic species on both sides of the same line [49].

Pokazalo se da korodiran OU utiče na biogeohemijske procese u životnoj sredini. Korodiran uranijum je jako redukciono sredstvo i ovo svojstvo, zajedno sa slobodnim vodonikom otpuštenom tokom korozije OU, smanjuje koncentraciju redukcionih agenasa (NO_3^-, Fe (III), SO_4^{2-}) [34,36,37,42,47]. Na primer, u vlažnim zemljištima i zalivskim sedimentima, prisustvo OU povećava stopu progresije kroz redoks niz (NO_3^-, Fe (III) i SO_4^{2-}) [47]. Procesom korozije smanjuje se koncentracija kiseonika, čije odsustvo je glavni preduslov pasivizacije korozione površine.

Uticaj bakterija i gljiva na koroziju OU

Negativan uticaj bakterija i gljivica na metale i legure je poznat. One doprinose ubrzanom procesu korozije ali i povećanju mobilnosti i bioraspoloživosti metalnih jona U u procesu korozije OU, a njihovo dejstvo uslovljeno je njihovom kontrolom disanja u različitim uslovima [51].

Do danas se samo jedna studija bavila istraživanjem uticaja bakterija na transformaciju metala OU [52]. U pomenutoj studiji, izolovani su aerobni i anaerobni mikroorganizmi iz zemljišta, bogata glinama, kao njihovim prirodnim staništima i u roku od 40 dana, pod različitim eksperimentalnim uslovima, praćen je njihov uticaj na korozionu stopu OU, ali i sa ciljem da se utvrde faktori dominacije različitih bakterijskih funkcionalnih grupa. Abiotički uslovi prouzrokovali su sličnu stopu korozije kao i u biotičkim sistemima, što potvrđuje dominaciju hemijskih parametara nad mikrobiološkim procesima. Pod aerobnim uslovima, proizvodi korozije su uglavnom bili u čvrstoj fazi (~85%), sa identifikovanim oblikom urana u vidu uraninita, šoepita i hernikovita (iz meta-autunit grupe). Korozija je manje obimna pod anaerobnim uslovima u poređenju sa aerobnim uslovima, uz gubitak mase posle 40 dana, oko 3%. Neefikasnost bakterija u doprinosu korozije OU je donekle iznenađujuća i u suprotnosti sa njihovim značajnim dejstvom na koroziju čelika ili gljivičnih uticaja na OU [53].

U opsežnim i jedinstvenim studijama medjusobnog dejstva gljiva i OU potvrdjeno je da prirodni mikroorganizmi i mycorrhizal gljive pokazuju visoku otpornost na prisustvo OU [54,55]. U ovim studijama, hife su dobile žutu boju što ukazuje na migraciju uranil jona u micelijumski sistem. Anaerobni uslovi doprineli su formiranju gljivičnog biofilma, koji zadržava vlagu na površini OU. Tokom 3 meseca inkubacije, u sistemu gljive – OU, utvrđen je gubitak težine od 5,5–8%, što se nije značajno razlikovalo od realnih uslova. Uzajamni mehanizmi delovanja, ukazuju da izloženost gliva dejstvu OU doprinose izlučivanju karboksilnih kiselina, naročito oksalne kiseline koja je poznata kao jak metalni helator. Ovaj rezultat je u skladu sa izuzetnom sposobnošću izloženih gljiva dejstvu OU da akumuliraju mobilne uranil jone svojom biomasom (300–400 mg g^{-1} suve mase), sa velikom biomineralizacijom u svim delovima gljivičnih kolonija. Elektronskom mikroskopijom i XRD analizom izvršena je karakterizacija biomase gljiva i dokazano je prisustvo stabilnih minerala uranil fosfata iz grupe meta autunita što potvrđuje da gljive transformišu metalni uranijum u termodinamički stabilne minerale [54,55].

TRANSPORT, MOBILNOST I BIORASPOLOŽIVOST RASPADA PRODUKATA OU

Transport OU aerosolom

Poznato je da se od 10–70% municije sa OU konvertuje u čestice aerosola prilikom kontakta sa tvrdom metom [14]. Disperzija čestica OU zavisi od njihove veličine i gustine i meteoroloških uslova [25]. Istraživanja u postkonfliktnim zonama na Kosovu potvrdila su da je najintezivnija kontaminacija OU bila u blizini mesta dejstva, da opada sa udaljenošću i da je bila merljiva od 10–50 m od mete [17]. U postkonfliktnoj zoni u Bosni i Hercegovini potvrđena je merljiva kontaminacija česticama OU na udaljenosti do 200 metara od tačke dejstva [26].

Istraživanja disperzije čestica oksida uranijuma, nastalih tokom sagorevanja otpadnog metala OU, u fabrici za proizvidnju OU u Colonie (NI, USA), procenjeno je, da je najmanje 3,4 t OU deponovano u okruženju fabrike na površini 1 km^2 a da su čestice OU detektovane na udaljenosti od 600 m od fabrike u pravcu dominantnog vetra. U prikupljenim česticama zemljišta i prašine identifikovani su mešoviti oksidi UO_2^{+x} i U_3O_8, 20–64 μm u prečniku [56,57].

U proseku se aerosoli rasprostiru do oko 5 km od mesta pogotka, mada je utvrđen njihov domet do 80 km od mesta pogotka U svakom slučaju, njihov dalji put je potpuno neizvestan, zavisi od ruže vetrova, vrste padavina i topografije terena [12].

Transport OU preko zemljišta i sedimenata

Poznavanje globalnog ciklusa urana nema za cilj samo određivanje nivoa kontaminacije i konstatovanje posledica, već sticanje saznanja uz pomoć kojih možemo sa sigurnošću predvideti sve procese koji utiču na njegov transport i fikscaciju i tako razviti modele zaštite životne sredine.

Mobilnost urana (pretežno U(VI) jona) u litosferi i hidrosferi odvija se u uslovima složenih hemijskih i fizičko–hemijskih prirodnih procesa. Rastvorljivost urana u zemljištu prvenstveno zavisi od pH sredine, redoks potencijala, a zatim od građe i mineralnog sastava čvrste faze, koncentracije neorganskih jedinjenja, količine i tipa organskih jedinjenja u zemljištima i zemljišnim rastvorima, temperature zemljišta, pritiska, sadržaja vlage i mikrobiološke aktivnosti. Transport urana u sistemu zemlja – voda odvija se uglavnom u rastvornom ili suspendovanom obliku, difuzijom ili masenim prenosom. Procesi koji uklanjaju uran iz zemljišnih rastvora su precipitacija, koprecipitacija, adsorpcija i ugrađivanje u biološke sisteme [58].

Utvrđeno je da zemljište, naročito ako je kiselo, može uticati na povećanje mobilnosti jona uranijuma, kao i na ravnotežu sorpcionih procesa. Autori su takođe proučavali kapacitet adsorpcije različitih tipova zemljišta i utvrdili da zemljišta, sa visokim sadržajem karbonata imaju najmanji adsorpcioni kapacitet, verovatno što grade veoma rastvoran uranil-karbonat, npr. UO_2CO_3, $[UO_2(CO_3)_2]$ ili $[UO_2(CO_3)_2]$. Pod aerobnim uslovima, gvožđe može igrati ključnu ulogu u kontrolisanju kretanja uranijuma kroz zemljište. Uranijum može kompleksirati gvožđe, koje se nalazi u mnogobrojnim solima u zemljištu. Takođe, uranijum gradi komlekse sa huminskim kiselinama prisutnim u zemljištu. Kompleksiranje sa organskim jedinjenjima utiče na smanjivanje brzine migracije uranijuma kroz zemljište čak za nekoliko reda veličine, tako da uranijum postaje značajno nepokretan [59].

Kvantifikacija sorpcije uranijuma na zemljišnim matriksima, može se odrediti primenom sekvencijalne ekstrakcije, korišćenjem različitih ekstrakcionih sredstava rastuće ekstrakcione moći, koja selektivno rastvaraju specifično asocirane frakcije urana iz istog geološkog uzorka. Tako se mogu odrediti vodo-rastvorljivi i izmenjivo-adsorbovani oblici urana, vezani za karbonatne, okside Fe i Mn, organsku fazu i strukturno vezan (inertan) [60]. Analiza zemljišta, kontaminiranih osiromašenim uranijumom, sa Kircudbright poligona, slabo snabdevena organskim materijama, pokazala je da je OU koloidno vezan sa gvoždjem i aluminijumom [61]. U sličnim studijama na zemljištima, niskog sadržaja organskih materija, u južnoj Srbiji, OU bio određen u frakcijama sa karbonatima i gvožđe/mangan oksidima [62].

Ispitivanjem migracije urana sa korodiranog penetratora u peskovito-glinovitim zemljištima Mojave Desert, region Yuma, u zavisnosti od dubine zemljišta, utvrđeno je da na površini zemljišta dominira žuto narandžasti šepiolit, na dubini od 2–4 cm, svetlo žuti metašopite a na dubini ispod 4 cm U je bio strukturno vezan za silikate [63]. Studija potvrđuje pokretljivost urana poreklom iz korozionih proizvoda penetratora kao i da se njegov oblik javljanja menja sa dubinom zemljišta. Tako je u zoni sukoba na Kosovu, nakon 1.5 godine od dejstva alijanse, kontaminacija uranom potvrđena na dubini od 20 cm [17], a u Bosni i Hercegovini, 6,5 godina nakon dejstva, na 40 cm dubine [26].

Eksperiment sa kolonama je korišćen je za ispitivanje mobilnosti produkarta korozije OU penetratora u trajanju od 98 dana. Utvrdjeno je prisustvo metašopita u interakciji sa mobilnim UO_2^{2+} u gornjih 1,7 cm kolone. Modeliranje je pokazalo da se većina rastvorenog U vezuje za frakciju peska sporom hemijsko kinetičkom desorpcijom i da će to ponašanje dominirati u realnim uslovima [42].

Schimmack i sar. sproveli su trogodišnja ispitivanja procesa korozije i luženja šest uzoraka ispaljene municije sa OU, mase od 145–264 g u laboratorijskim uslovima u koloni mešanjem sa 3.3.kg različitih tipova zemljišta [45]. Kolone su se kvasile jednom nedeljno sintetičkom kišom, a sadrzaj urana odredjivan u procednim vodama. Sintetička kiša sadržala je 0,09 mM NH_4NO_3 ,0,08 mM $(NH_4)_2SO_4$ i 0,05 mM $CaSO_4$, pH 6. Dobijeni rezultati ukazuju da je u proseku oko 14,5 g ili 7,9% od inicijalne mase izluženo sa dramatičnim povećanjem u trećoj godini u odnosu na prvu što predstavlja potencijalnu opasnost za kontaminaciju podezmnih voda, ali i vremensku promenljivost i nezakonitost. Prosečno se izluživalo oko 1mg U nedeljno što predstavlja potencijalnu opasnost za kontaminirane lokalitete imajući u vidu da je 15 μg L^{-1} preporuka WHO za dozvoljen sadržaj urana u vodi za piće. Utvrdjeno je da zemljišta sa visokim sadržajem peska, imaju manji sorpcioni kapacitet za uran što je slučaj na većini kontaminiranih lokaliteta. Uranske vrste u ocednim vodama, zemljištu i produktima korozije na površini OU određivane su lasersko indukovanom fluorescentnom spektroskopijom (TRLFS). Dominantne vrste uranskih jedinjenja u ocednim vodama su bili hidroksidni kompleksi $(UO2)_3(OH)_5^+$ u nekarbonatnim zemljištima i karbonatni komleksi u karbonatnim zemljištima $UO_2(CO_3)_3^{4-}$. Najdominantniji faktor u formiranju uranskih komleksa i vrsta u zemljišnim rastvorima je pH, koji se za vreme trajanja ogleda kretao izmedju 6 i 8 [64]. Na kraju ogleda ispitani su uzorci zemljišta koji okružuje OU, sa različitih dubina i OU. Utvrđena je razlika izmedju početne i završne mase. Produkti korozije, crne i žute boje, uklonjeni su mehanički a potpuno čišćenje metaka od produkata korozije obavljeno je rastvaranjem u 65% azotnoj kiselini, pri čemu su se površinski produkti korozije brzo rastvorili za razliku od metalnog uranijuma. TRLFS metodom u zemljištu su detektovani minerali $AlH(UO_2)(PO_4)_4\ 16H_2O$ (sabugalit) i autunit $Ca(UO_2)_2(PO_4)_2\ 10(H_2O)$. Oko 75% uranijuma je detektovano u zemljištu u samoj blizini projektila, 12% je pronadjeno u ostatku zemljišta, 13% je migriralo niz zemljišni profil. Na zemljištima tipa luvisol sa šljunkovitim frakcijama, konstatovano je 2–3% urana iznad metalnog fragmenta kao posledica zemljišno-vodnog potencijala što je prouzrokovalo kretanja vode na gore. U produktima korozije na osiromešenom uranijumu konstatovane su i druge uransko-fosfatne vrste, kao što je UO_2HPO_4.

Saradnici Instituta za tehnologiju nuklearnih i drugih mineralnih sirovina verifikovali su tehničko-tehnološko rešenje, URANOFIX, namenjeno remedijaciji zemljišta kontaminiranog osiromašenim uranijumom na bazi domaćih fosfata ležišta Lisina „Bosilegrad“ bazirajući se na činjenici da se uran u prirodi pojavljuje u mineralima na bazi fosfora. Fosfatno indukovana stabilizacija, primenom apatita, zasnovana je na mehanizmu precipitacije nove faze, tj. apatit se rastvara i formira se nova faza uranijum fosfata. Niska rastvorljivost autunuta $K_{sp} = 10^{-49}$ je osnov njihove stabilnosti u dugom geološkom periodu (milioni godina) pod veoma različitim uslovima, pH 2 do 12 [58].

Biodostupnost: adsorpcija od strane biljaka i prostim organizmima

Biljke i drveće

Postoji veliki broj istraživanja na temu usvajanja urana antropogenog porekla, od strane gajenih biljaka. Sklonost pojedinih biljnih vrsta da usvajaju uran preko fiziološkog praga (hiperakumulatori) iskorišćen je za fitoremedijaciju kontaminiranih medijuma uranom [1,6,9,10,11,58,65]. Relativno je malo studija o usvajanju OU od strane biljaka. Istraživanja Schimmack i sar. i Oliver i sar., ilustruju toksičnost OU na biljke, trave i paprat, uzorkovanih sa streljačkog poligona Kirkcudbright i Eskmeals u Velikoj Britaniji. Zabeležena maksimalna kontaminacija je 3,38 mgU kg^{-1} suve mase biljaka [45,66].

Efekat usvajanja produkata korodiranog penetratora OU od strane tri vrste trava, (*Schizachirium Scoparium*, *Buchloe Daktiloide i Aristida Purpurea*) je bio ispitivan u laboratorijskim uslovima [67]. *A. purpurea i B. dactiloides* pokazivale su otpornost do koncentracije OU u zemljištima od 25000 mg kg^{-1}, kada se fitotoksično dejsto urana manifestovalo kroz smanjenje biomase. Rast *S. Scoparium* je bio stimulisan na zemljištu sa koncentracijama urana od 50 i 500 mg kg^{-1} što je potvrdjeno i sa biljkama kukuruza pri koncentarciji od 25 mg kg^{-1} u vegetacionim ogledima [65].

OU je takođe otkriven u crnim hrastovima, koji su rasli na močvarnim lokalitetima kontaminiranim osiromašenim uranijumom. Koncentracije U u stablu biljke su merene preko 10 godina i ustanovljeno je da je koncentracija ekvivalentna koncentracijama podzemnih voda. Autori sugerišu, da se hrast koristi kao bioindikator kontaminacije različitih medijuma sa OU [68].

Zbog bliskog jonskog radijusa (r = 1.05 Å) sa radijusom Ca^{2+} (r = 1.06 Å) uran zamenjuje kalcijum. Iz tog razloga sa stanovišta dislokacije urana iz zemljišta u biljku, kalcijum se smatra metaboličkim analogom urana [69].

Lišajevi , mahovine i gljive

Analiza lišaja kore drveta, obezbeđuje korisnu procenu kvaliteta vazduha, jer oni akumuliraju supstance iz atmosferskih čestica. Povišen sadržaj OU u lišajevima je konstatovan na lokacijama u Crnoj Gori i Bosni i Hercegovini, što se pripisuje vazdušnom zagađenju [17,25,26].

U regionu zapadnog Balkana, sa visokim aktivnostima ^{238}U, od 1130–5390 Bk kg^{-1}, potvrđen je visok nivo aktivnosti u mahovini, lišajevima i gljivama [70]. Lišaji, mahovine i gljive su jeftini i lako dostupni bioindikatori nivoa kontaminacije medijuma sa OU [71].

Gliste

Oko 80% ukupne faune u zemljištu čine gliste, koje efikasno akumuliraju teške metale i radionuklide i igraju značajnu ulogu u lancu ishrane. U prirodno visokom sadržaju uranijuma u Norveškoj, konstatovan je visok transfer faktor uranijuma (0,09–0,25) iz zemljišta u različite vrste glista [72]. Oliver i sar., su u prikupljenim glistama sa kontaminiranih poligona sa OU, Kirkcudbright i Eskmeals u Velikoj Britaniji, konstatovali visok stepen akumulacije, organski vezanog urana u odnosu na prirodni nivo [66]. Rezultati su u suprotnosti sa onima dobijenim sa Kosova, gde nisu utvrđene razlike što se pripisuje heterogenosti zemljišta i kraćim vremenom izlaganja glista uranu [73].

ZAKLJUČAK

Cilj rada je davanje doprinosa razumevanju procesa i faktora koji kontrolišu koroziju osiromašenog uranijuma i transport produkata korozije penetratora kroz životnu sredinu.

Formiranje aerosola OU tokom interakcije penetratora OU sa čvrstom metom, predstavlja dominantan put kontaminacije životne sredine vazdušnim transportom i glavna opasnost na ljudsko zdravlje. Put kontaminacije ovih čestica pre svega zavisi od atmosferskih prilika.

Korozija penetratora OU je složen proces, specifičan za svaki lokalitet i odvija se u interakciji hemijskih, fizičko–hemijskih i biogeohemijskih procesa.

Dominatni faktori koji utiču na koroziju su pH sredine, redoks potencijal, geomorfološka građa zemljišta, koncentracija i vrste neorganskih i organskih jedinjenja u zemljištima, temperatura zemljišta, pritisk, sadržaj vlage, mikrobiološke i fungicidne aktivnosti, mikro zemljišne faune, salinitet (ukoliko je penetrator dospeo u morske vode). Isti faktori utiču i na rastvaranje produkata korozije. Zaključak je, da svaki kontaminirani lokalitet predstavlja specifičan slučaj i zahteva obiman monitoring i multidisciplinaran pristup saniranja.

U uverenju da na temu zaštite stanovništva od jonizujućeg zračenja nikada nije previše rečeno, izložen materijal predstavlja i svojevrsnu deklaraciju tehnološkog pokajanja, priznanje da je razvojni put ugrožen ozbiljnim neplaniranim efektima i ujedno apel da se odnos prema životnoj sredini i stanovništvu vrati iz zloupotrebe u oblast razumne upotrebe. Naš zadatak je da se pogubno dejstvo osiromašenog uranijuma minimizira i da se spreči njegovo prodiranje u lanac ishrane, u protivnom ” *invisible threat*” će delovati beskonačno dugo sa svim svojim pogubnim posledicama na buduće generacije.

Zahvalnica

Autori su zahvalni Ministartvu prosvete, nauke i tehnološkog razvoja Republike Srbije koji su podržali ova istraživanja kroz Projekat br. TR 31003.

LITERATURA

[1] M. Stojanović, J. Milojković, in: Ivan Golubev (Eds.), Phytoremediation of Uranium Contaminated Soils, Handbook of Phytoremediation Nova Science Publishers Inc., New York, United States of America, 2011, pp. 93––136.

[2] V. Yablokov, B.Vassily, B. Nesterenko, V. Nesterenk, Chernobyl Consequences of the Catastrophe for People and the Environmen, Published by Blackwell Publishing on behalf of the New York Academy of Sciences, Boston, MA, 2009.

[3] http://www.world-nuclear.org/info/Safety-and-Security/Radiation-and-Health/Naturally-Occurring-Radioactive-Materials-NORM/#.UknmEYbIYvk (18.9.2013).

[4] http://www.epa.gov/radiation/tenorm/ (18.9.2013).

[5] I. Grzetic, R. Jelenković, Prirodni radioaktivni elementi: geolosko poreklo, oblici pojavljivanja i migracije, Jonizujuća zračenja iz prirode, JDZZ Beograd (1995) 3–39.

[6] M. Stojanović, J. Milojković, Z. Lopičić, M. Mihajlović, M. Rajković, G. Vitorović, in: Alik Ya, Vasiliev and Mikhail Sidorov (Eds.), Anthropogenic sources of uranium in Serbia – risk assessment on environment and human health. Uranium: Characteristics, Occurrence and Human Exposure, Nova Science Publishers Inc., New York, 2012, pp 46–86.

[7] G.D. Djurić, Lj.D. Popović, Uranijum u životnoj sredini - Nulto stanje u Srbiji, Hem. Ind. **55** (2001) 289–294.

[8] M. Stojanović, J. Mrdaković Popić, D. Stevanović, Lj. Martinović, Phosphorus fertilizers as source of uranium in Serbian soils, Agron. Sustain. Dev. **26** (2006) 179–183.

[9] M. Stojanović, M. Mihajlović, J. Milojković, Z. Lopičić, M. Adamović, S. Stanković, Efficient phytoremediation of uranium mine tailings by tobacco, Environ. Chem. Let. (2012) 377–381.

[10] M. Sarić, M.Stojanović, M.Babić, Uranium in plant species grown on natural barren soil. J Plant. Nutr. **18** (1995) 1509–1518.

[11] M. Stojanović, D. Stevanović, D. Iles, M. Grubišić, J. Milojković, The effect of the uranium content in the tailings on some cultivated plants, Water Air Soil Poll. **200** (2009) 101–108.

[12] M. Rajković, Osiromašeni uranijum, I-Uranijum, radioaktivnost i zakonska regulativa, Hem. Ind. **55** (2001) 167–182.

[13] M. Rajković, Depleted Uranium, S. Brstina, Ed., Vojnoizdavački zavod, Beograd, 2001.

[14] A. Bleise, P.R. Danesi, W. Burkart, Properties, use and health effects of depleted uranium (DU): A general overview. J. Environ. Radioactiv. **64** (2003) 93–112.

[15] E.I. Hamilton, Depleted uranium (DU): a holistic consideration of DU and related matters. Sci. Total Environ. **281** (2001) 5–21.

[16] S. Handley-Sidhua, M. Keith-Roach, R. Loyd, J. David, A.Vaughan, A review of the environmental corrosion, fate and bioavailability of munitions grade depleted uranium. Sci Total Environ, **408** (2010) 5690–5700.

[17] UNEP, Depleted uranium in Kosovo, post-conflict environmental assessment. United Nations Environment Programme, Switzerland, 2001, http://postconflict.unep.ch/publications/uranium.pdf (18.9.2013).

[18] S.Z. Žunić, J.W. Mietelski, S. Blazej, P. Gaća, E. Tomankiewicz, P. Ujić, I. Celiković, O. Cuknić, M. Demajo,Traces of DU in samples of environmental bio-monitors (non-flowering plants, fungi) and soil from targetsites of the Western Balkan region, J. Environ. Radioactiv. **99** (2008) 1324–1328.

[19] M. B. Rajković, A. Đorđević, in: M. Stojanović (Ed.), Possibility of serbian soil contamination by depleted uranium after NATO bombing 1999, Radionuclide contamination of Serbian soil and remediation possibility, ITNMS,Belgrade, 2006, pp. 167–219.

[20] UNEP, Technical report on capacity-building for the assessment of depleted uranium in Iraq, Switzerland: United Nations Environmental Program, 2007.

[21] G. Jia, M. Belli, U. Sansone,S. Rosamilia, S. Gaudino, Concentration and characteristics of depleted uranium in biological and water samples collected in Bosnia and Herzegovina, J Environ. Radioactiv. **89** (2006) 172–187.

[22] C. Papastefanou, Depleted uranium in military conflicts and the impact on the environment, Health Phys. **83** (2002) 280–282.

[23] R. L. Erikson, A review of the environmental behavior of uranium derived from depleteduranium alloy penetrators, Technical Report PNL-7213, Pacific Northwest Laboratory, Richland, Washington DC,1990.

[24] M. Radenković, T. Anđelić, M. Kovačević, P.Vukotić, Depleted uranium in the air during the clenup operations of Cape Arza, J. Serb. Chem. Soc. **69** (2004) 1153–1155.

[25] UNEP, Depleted Uranium in Serbia and Montenegro-Post Conflict Environmental Assessment, Geneva, 2002. http://postconflict.unep.ch/publications/duserbiamont.pdf (18.9.2013).

[26] UNEP, Depleted uranium in Bosnia and Herzegovina: Postconflict environmental assessment. United Nations Environment Programme, Imprimerie Chirat, 2003. http://www.unep.org/disastersandconflicts/portals/155/disastersandconflicts/docs/dup/BiH_DU_report.pdf (18.9.2013).

[27] UNEP, Discussion of health issues: appendix K. In: Depleted Uranium in Serbia and Montenegro – Post Conflict Environmental Assessment. UNEP, Geneva, 2002, pp. 179–183.

[28] S. Petković, M. Zarić, Z. Dević, Upotreba municije sa osiromasenim uranom u agresiji NATO na saveznu republiku Jugoslaviju, Hem. Ind. **55** (2001) 318–324.

[29] P.R. Danesi, A. Markowicz, E. Chinea-Cano, W. Burkart, B. Salbu, D. Donohue, Depleted uranium particles in selected Kosovo samples, J. Environ. Radioact. **64** (2003) 143–154.

[30] B. Salbu, K. Janssens, O. Lind, K. Proost, P. Danesi, Oxidation states of uranium in DU particles from Kosovo, J. Environ. Radioact. **64** (2003) 167–173.

[31] B. Salbu, K. Janssens, O. Lind, K. Proost, P. Danesi, Oxidation states of uranium in depleted-uranium particles from Kuwait, J. Environ. Radioact. **78** (2005) 125––135.

[32] M.A. Parkhurst, Measuring aerosols generated inside armoured vehicles perforated by depleted uranium ammunitions, Radiat. Prot. Dosim. **105** (2003) 167–170.

[33] W. Dong, G. Xie, T.R. Miller, M.P. Franklin, T.R. Oxenberg, E.J. Bouwer, Sorption and bioreduction of hexavalent uranium at a military facility by the Chesapeake Bay, Environ. Pollut. **142** (2006) 132–142.

[34] C.A. Laue, D. Gates-Anderson, T.E. Fitch, Dissolution of metallic uranium and its alloys, J. Radioanal. Nucl. Chem. **261** (2004) 709–717.

[35] M.J.M. Pourbaix, Atlas of Electrochemical Equilibria in Aqueous Solutions, (Eds.), National Association of Corrosion Engineers (NACE), Houston, TX, 1974.

[36] S. Handley-Sidhu, The biogeochemical controls on the corrosion and fate of depleted uranium, PhD Thesis, University of Plymouth, 2008.

[37] S. Handley-Sidhu, P.J. Worsfold, C.Boothman, J.R. Lloyd, R. Alvarez, F.R. Livens, Corrosion and fate of depleted uranium penetrators under progressively anaerobic conditions in estuarine sediment, Environ. Sci. Technol. **43** (2009) 350–355.

[38] C.C. Toque, A.C. Baker, MOD DU program: The corrosion of depleted uranium in the Kirkcudbright and Eskmeals terrestrial environments. DSTL/CR10978 V2.0, Alverstoke, 2006.

[39] D. Langmuir, Uranium solution-mineral equilibria at low temperature with applications to sedimentary oredeposits, Geochim. Cosmochim. Acta **42** (1978) 547––569.

[40] P.P. Trzaskoma, Corrosion rates and electrochemical studies of depleted uranium alloy tungsten fiber metal matrix composite. J. Electrochem. Soc. **129** (1982) 1398––1402.

[41] J.F Mcintyre, E.P. Lefeave, K.A. Musselman, Galvanic corrosion behaviour of depleted uranium in synthetic seawater coupled to aluminium, magnesium, and mild steal, Corros. Sci. **44** (1988) 502–510.

[42] S. Handley-Sidhu, N.D. Bryan, P.J. Worsfold, D.J. Vaughan, F.R. Livens, M.J. Keith-Roach Corrosion and transport of depleted uranium in sand-rich environments, Chemosphere **77** (2009) 1434–1439.

[43] M. Fomina, J. Charnock, S. Hillier, R. Alvarez, G.F. Livens, M.G. Gadd, Role of fungi in the biogeochemical fate of depleted uranium, Curr. Biol. **18** (2008) 375–377.

[44] N. Baumann, T. Arnold, G. Geipel, R.E. Trueman, S. Black, D. Read, Detection of U(VI) on the surface of latered depleted uranium by time-resolved laser-induced fluorescence spectroscopy (TRLFS), Sci. Total. Environ. **366** (2007) 905–909.

[45] W. Schimmack, U. Gerstmann, W. Schultz, G. Geipel, Long-term corrosion and leaching of depleted uranium (DU) in soil, Radiat. Environ. Biophys. **46** (2007) 221––227.

[46] E. Mattsson, Basic Corrosion Technology for Scientists and Engineers, Ellis Horwood Limited, Chichester, 1989.

[47] S. Handley-Sidhu, P. Worsfold, F.R. Livens, D. Vaughan, J. Lloyd, C. Boothman et al. Biogeochemical controls on the corrosion of depleted uranium alloy in subsurface soils, Environ. Sci. Technol. **43** (2009) 6177–6182.

[48] J.M. Senko, J.D. Istok, J.M. Suflita, L.M. Krumholz, In-situ evidence for uranium immobilization and remobilization, Environ. Sci. Technol. **367** (2002) 1491–1496.

[49] N. De Zoubov, Establishment and interpretation of potential-pH equilibrium diagrams.M. Pourbaix (Ed.), Atlas of Electroch. Equilibria in Aqueous Solution, NACE, Houston, TX. 1974, pp. 198–212.

[50] M. Stojanović, Č. Lačnjevac, M. Lopičić, M. Rajković, M. Petrović, Corrosion and corrosion behavior of depleted uranium, In: Korozija i zaštita materijala, Izdavač: ITNMS Beograd i Inženjersko društvo za koroziju Beograd, Z. Gulišija i Č. Lačnjevac, ur., 2012, str. 223–261.

[51] G.D. Gadd. Metals, minerals and microbes: Geomicrobiology and bioremediation, Microbiology **156** (2010) 609–643.

[52] R. Alvarez, F.R. Livens, J. Lloyd, J. Holt, C. Boothman, W. Wincott P et al. Geochemical and microbial controls on the decomposition of depleted uranium in the environment: Experimental studies using soil microorganisms. Geomicrobiol. J. **28** (2011) 457–470.

[53] H.A. Videla, K. Herrera, Microbiologically influenced corrosion: Looking to the future, Int. Microbiol. **8** (2005) 169–180.

[54] M. Fomina, J. Charnock, S. Hillier, R. Alvarez, G.F. Livens, M.G. Gadd, Role of fungi in the biogeochemical fate of depleted uranium, Curr. Biol. **18** (2008) 375–377.

[55] M. Fomina, J. Charnock, S. Hillier, R. Alvarez, G.F. Livens, M.G Gadd, Fungal transformations of uranium oxides, Environ. Microbiol. **9** (2007) 1696–1710.

[56] S.N. Lloyd, S.R. Chenery, R.R. Parrish, The distribution of depleted-uranium contamination in Colonie, New York, Sci. Total. Environ. **408** (2009) 397–407.

[57] S.N. Lloyd, S.R.Chenery, R.R.Parrish, The morphologies and compositions of depleted-uranium particles from an environmental case study, Mineral. Mag. **73** (2009) 495––510.

[58] M. Stojanović, Radionuclide contamination of Serbian soil and remediation possibility, Institute for Technology of Nuclear and Other Minerals Raw Materials, Belgrade, 2006.

[59] M.C. Duff, J.U. Coughlin, D.B. Hunter, Uranium coprecipitation with iron oxide minerals, Geochim. Cosmochim. Act. **66** (2002) 3533–3547.

[60] A. Tessier, M.P. Campbell, M. Bisson, Sequential extraction procedure for speciation of Particulate Trace Metals, Anal. Chem. **54** (1979) 844–851.

[61] C.M. Graham, W.I. Oliver, B.A. MacKenzie, M.R. Ellam, G.J. Farmer, An integrated colloid fractionation approach applied to the characterisation of porewater uranium–humic interactions at a depleted uranium contaminated site, Sci. Total. Environ. **404** (2008) 207––217.

[62] M. Radenković, S. Cupac, J. Joksić, D. Todorović, Depleted Uranium mobility and fractionation in contaminated s oil (Southern Serbia), Env. Sci. Pollut. Res. **15** (2008) 61–67.

[63] B. Buck, A.L. Brock, W.H. Jonhso, A.L. Ulery, Corrosion of Depleted Uranium in an Arid Environment: Soil-Geomorphology, SEM/EDS, XRD, and Electron Microprobe Analyses, Soil and Sediment Contamination: An International Journal **13** (2004) 545–561.

[64] G. Echevarria, M. Sheppardi, J. Morel, Effect of pH on the sorption of uranium in soils, J. Environ. Radioact. **53** (2001) 257–264.

[65] M. Stojanović, D. Stevanović, D. Iles, M. Grubišić, J. Milojković, Phytotoxic effect of uranium on the growing up and development plant of corn, Water Air Soil Poll. **209** (2010) 401–410.

[66] W.I. Oliver, M.C. Graham, B.A. MacKenzie, M.R. Ellam, J.G. Farmer, Assessing depleted uranium (DU) contamination of soil, plants and earthworms at UK weapons testing sites, J. Environ. Monit. **9** (2007) 740–748.

[67] C.M. Meyer, T. McLendon, D. Price, J. Fleckenstein, E. Schnug, Uptake of munition-derived depleted uranium by three grass species. J. Plant. Nutr. **27** (2004) 1415––1429.

[68] D.J. Edmands, J.D. Brabander, D.S. Coleman, Uptake and mobility of uranium in black oaks: Implications for biomonitoring depleted-uranium-contaminated groundwater, Chemosphere **44** (2001) 789–795.

[69] H. Tunney, M. Stojanović, J. Mrdaković Popić, D. McGrath,ʹ C. Zhang, Relationship of soil phosphorus with uranium in grassland mineral soils in Ireland using soils from a long-term phosphorus experiment and a National soil database, J. Plant. Nutr. Soil Sci. **172** (2009) 346–352.

[70] S. Žunić, J. Mietelski, S. Blazej, P. Gaća, E. Tomankiewicz, P. Ujić, I. Celiković, O. Cuknić, M. Demajo, Traces of DU in samples of environmental bio-monitors (non-flowering plants, fungi) and soil from target sites of the Western Balkan region, J. Environ. Radioactiv. **99** (2008) 1324–1328.

[71] A. Di Lella, L. Frati, G. Protano, F. Riccobono, Lichens as biomonitors of uranium and other trace elements in an area of Kosovo heavily shelled with depleted uranium rounds, Atmos. Environ. **37** (2003) 5445–5449.

[72] J. Mrdakovic Popic, J. Brit Salbu, L. Skipperud, Ecological transfer of radionuclides and metals to free-living earthworm species in natural habitats rich in NORM, Sci. Total. Environ. **14** (2011) 167–176.

[73] A. Di Lella, F. Nannoni, G. Protano, F.Riccobono, Uranium contents and 235U/238U atom ratios in soil and earthworms in western Kosovo after the 1999 war, Sci. Total. Environ. **337** (2005) 109–118.

SUMMARY

ECOLOGICAL AND CORROSION BEHAVIOR OF DEPLETED URANIUM

Mirjana D. Stojanović[1], Časlav M. Lačnjevac[2], Marija L. Mihajlović[1], Marija V. Petrović[1], Tanja D. Šoštarić[1] Jelena T. Petrović[1], Jelena V. Milojković[1]

[1]Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia
[2]Faculty of Agriculture, University of Belgrade, Serbia

(Review paper)

Environmental pollution with radionuclides, particularly uranium and its decay products is a serious global problem. The current scientific studies estimated that the contamination originating from TENORM, caused by nuclear and non-nuclear technologies, has significantly increased natural level of radioactivity in the last thirty years. During the last decades all the more were talking about the “new pollutant” – depleted uranium (DU), which has been used in anti-tank penetrators because of its high density, penetration and pyrophoric properties. It is estimated that during the Gulf War, the war in Bosnia and Yugoslavia and during the invasion of Iraq, 1.4 million missiles with depleted uranium was fired. During the NATO aggression against the ex Yugoslavia in 1999, 112 locations in Kosovo and Metohija, 12 locations in southern Serbia and two locations in Montenegro were bombed. On this occasion, approximately 10 tons of depleted uranium were entered into the environment, mainly on land,where the degree of contamination ranged from 200 to 235000 Bq/kg, which is up to 1000 times higher than the natural level. Fourteen years ago there was very little information about the behavior of ecological systems damaged by DU penetrators fired. Today, unfortunately, we are increasingly faced with the “invisible threat" of depleted uranium, which has a strong radioactive and hemotoxic impact on human health. Present paper provides a detailed overview of the current understanding of corrosion and corrosion behavior of DU and environmental factors that control corrosion, together with indicators of environmental impact in order to highlight areas that need further attention in developing remediation programs.

Keywords: Depleted uranium • Corrosion • Behavior • Environment

Basic chemical composition of meat and carcass quality of fattening hybrids with different slaughter weight

Dragomir R. Lukač[1], Vitomir S. Vidović[1], Aleksandar Lj. Stoisavljević[2], Nikola M. Puvača[1], Natalija R. Džinić[3], Vladimir M. Tomović[3]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Novi Sad, Serbia
[2]PTK Panonija a.d., BačkaToplola, Serbia
[3]University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

SCIENTIFIC PAPER

UDC 636.4.084.637.5.04

Hem. Ind. **69** (2) 121–126 (2015)

doi: 10.2298/HEMIND131128027L

Abstract

Meat is a very rich and universal source of nutrients. The primary importance of meat is based especially on protein content. Optimum slaughter weight has influence on the basic chemical composition of meat. Fattening pigs were classified by their weight into three groups before the slaughter day. The highest content of proteins and water was found in the part of ham and shoulder in the group of animals at 100–110 and 111–120 kg of live weight, respectively, while the lowest content was found in the part of neck in animals at 121–130 kg of live weight. The highest content of intramuscular fat and mineral substances was in animals at 121–130 kg (neck and loin), while the lowest content was in animals at 100–110 kg live weight (ham). The pH values were within the optimum limits (5.33–5.77). Differences between the groups for the values of proteins content, mineral substance and pH values were statistically highly significant ($p < 0.05$; $p < 0.01$), while for the contents of water and intramuscular fat there were no differences ($p > 0.05$) between the groups.

Keywords: fattening, pigs, slaughter weight, chemical composition, meat.

Meat quality is attracting increasingly more attention in pig breeding. Quality of the carcass and meat are influenced by genetic and many paragenetic factors [1,2], including the final body weight of fattening pigs. In Serbia, pigs are slaughtered at approximately 105––110 kg of body weight, and thus there is industry pressure to increase slaughter weight in order to enable Serbian pork to be more competitive on both domestic and international market. Production of pig meat is based on crossbreds, *i.e.*, hybrid pigs. In crossbreeding in Serbia, the main pig breeds for the dame line are Yorkshire and Swedish Landrace, and lately also German Landrace. Duroc and German Landrace are usually used as a sire line. Over the years, genetic selection has greatly improved the growth rate of pigs. However, little is known about the combined or interactive effect of growth rate and final body weight on pig carcass and meat quality characteristics. The consumers' interest in pork and pig products is maintained and based upon many factors. Composition of pork and there by its nutrition value, sensory perception, especially its fine taste, softness, crispness and juiciness, together with a variety of culinary preparations may serve as the examples.

Consumers consider that a quality meat is the meat which has an optimum composition regarding its nutritive value. Meat is a very rich and universal source of nutrients. Primary importance of meat is based especially on protein content [3]. Gonzalez *et al*. [4] and Tibau *et al*. [5] studied chemical composition of pork in relation to slaughter value, gender and genotype. Chemical composition of pork was analysed in final hybrids of slaughtered animals in weight intervals between 25 and 140 kg of body weight. As presented by Fortin [6], slaughter weight influences its chemical composition more than physical indicators. These conclusions are confirmed by investigation of Bečkova [7], who monitored the percentage of intramuscular fat in 78 final hybrids with a view to various slaughter weights. Mikule [8] studied the optimum slaughter weight influencing the basic chemical composition of meat, also confirming the conclusions presented above.

Increasing slaughter weight has the advantage of reducing the overhead costs for producers, slaughterers and processors by increasing carcass yields, improving meat to bone ratio and reducing chilling and processing losses [9]. Furthermore, at heavier weights, pork quality was rated higher in terms of juiciness, flavour and tenderness [10], which, according to Hugo et al. [11], would be attributable to differences in intramuscular fat composition. However, it has also been shown that each 10 kg increase from 100 kg of live weight leads to a slightly lower average daily gain, sig-

---

Correspondence: D. Lukač, University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.
E-mail: dragomir.lukac@stocarstvo.edu.rs

Paper received: 28 November, 2013
Paper accepted: 27 March, 2014

nificant deterioration in feed efficiency, reduced lean deposition and poorer meat quality [12].

The use of modern, high-lean growth potential genotypes may represent a valid approach, as in theory they could be taken to heavier weights without compromising growth performances and carcass and meat quality traits. By determining meat quality of pigs with different body weight at slaughter, we could be able to determine the optimal fattening time which directly affects the cost of production, but also to review the selection criteria which are applied in pure breeds, as well as the effects of the selection. The aim of this paper was to evaluate the effects of heavier slaughter weight on carcass and meat quality of pigs reared on a farm in Serbia.

MATERIAL AND METHODS

Animal trials

For the purposes of this investigation a total of 40 pigs of different crossbreds were used, obtained by mating F_1 generation sows from boars of Landrace and Yorkshire. Before the slaughter, the fatlings were classified into three weight groups: 100–110 kg (14 fatlings), 111–120 kg (13 fatlings) and 121–130 kg (13 fatlings). The composition and quality of used feed mixtures in fattening was the same for all animals. Pigs were not fed 12 h prior to slaughtering.

Sample collection

After the primary slaughtering process, right and left halves were cut into main parts and into parts of less value (head, glands, legs, tail and kidneys). Carcass dissection was performed on the model of EU 1992. Samples for chemical analysis were taken from four places of the carcass (neck, loin, shoulder and ham).

Chemical analyses

Chemical analyses based on determination of the content of proteins, water, intramuscular fat and mineral substances were conducted according to methods recommended by International Organization for Standardization [13,14]. The pH meter was calibrated before and during the readings using standard phosphate buffers and adjusted to the expected temperature of measured muscles [15]. All the analyses were performed in duplicate.

Statistical analyses

All the data are presented as average values and standard deviation. Independent *t*-test and analysis of variance (Duncan's multiple range tests) were used to test the hypothesis about differences between two or more average values. The software package Statistica 12 was used for the analysis.

RESULTS AND DISCUSSION

The Table 1 presents parameters of the quality value of carcass of fatlings according to the slaughter weight. The average weight of pigs from 100 to 110 kg at slaughter was 106.85 kg, for pigs from 111 to 120 kg it was 113.83 kg and for pigs from 121 to 130 kg it was 127.40 kg. As observed by other researchers [3], there were increases in worm carcass and the percentage of meat in carcass weight when the body weight of pigs at slaughter increased. The optimal final body weight of fattening depends on the customer requirements, needs of manufacturing industry, cost of production, as well as the genetic potential of pigs for meat production [16].

The data in the Table 2 present the proportion of major parts in the pork carcass of fatlings according to the slaughter weight. The largest and most important part in the carcass is ham, with a share of about 31% of complete pig carcass. After ham, the front third (neck, blade and shank) makes up the largest share of the carcass, about 26%, followed by ribs with bacon about 16% and loin about 15% in all groups of fatlings. Similar shares in the carcass were reported [12,17,18]. In the present study, the slaughter weight did not influence carcass composition of pigs of different body weight. In research of Correa et al. [19] even though the weight of the shoulder increased with slaughter weight, only the yield of ham was affected by body weight. Latorre *et al.* [12] also reported a higher shoulder weight with increased slaughter weight, although there was no observed effect on yield.

Table 1. Parameters of the quality value of carcass of fatling according to the slaughter weight; $\overline{X} \pm S_\mathrm{d}$, $\overline{X}$ *– mean value;* S_d *– standard deviation*

Parameter	Slaughter weight, kg		
	100–110	111–120	121–130
Slaughter weigh, kg	106.85±1.24	113.83±1.85	127.40±1.24
Weight of hot carcass sides, kg	86.14±1.75	90.16±1.35	103.20±1.11
Weight of cold carcass sides, kg	82.15±1.52	85.38±1.33	98.75±1.36
Losscooling, %	3.28±0.50	4.13±1.18	3.46±0.96
Randman, %	80.57±0.90	77.91±0.79	80.96±0.41
Content of meat in the carcass, %	46.24±1.15	48.15±1.08	55.34±1.74

Table 2. Proportion of major parts in the pork carcass of fatling according to the slaughter weight; $\overline{X}$ – mean value; S_d – standard deviation

Parameter	Slaughter weight, kg					
	100–110		111–120		121–130	
	$\overline{X}\pm S_d$	%	$\overline{X}\pm S_d$	%	$\overline{X}\pm S_d$	%
Head, kg	3.87±0.05	4.50	4.01±1.56	4.70	4.76±0.16	4.82
Anterior legs, kg	0.81±0.01	0.90	0.87±0.03	1.02	1.00±0.08	1.02
Posterior legs, kg	1.05±0.02	1.19	1.11±0.08	1.30	1.21±0.13	1.22
Jowl, kg	2.53±0.11	3.14	2.53±0.24	2.96	3.06±0.02	3.10
Loin, kg	12.18±0.45	15.25	13.68±0.77	16.02	14.52±0.62	14.71
Fillet, kg	0.71±0.04	0.72	0.90±0.06	1.05	0.93±0.05	0.94
Third of front (neck, blade, shank), kg	21.90±0.45	26.75	22.48±1.03	26.33	25.70±0.67	26.04
Ham, kg	26.09±0.37	31.31	25.63±0.53	30.01	30.52±0.65	30.92
Ribs with bacon, kg	12.98±0.42	16.23	14.15±0.37	16.57	16.97±0.73	17.19

Results of the basic chemical analyses are presented in the Tables 3–7. The average values of proteins are presented in the Table 3. The highest average values of crude proteins were assessed in animals at 100–110 kg of live weight (loin, 22.54%), while the lowest average values were found in animals at 121–130 kg of live weight (neck, 17.43%). According to Okrouhlá *et al*. [20], the content of proteins in clean lean ranged between 18 and 22%. The highest average values of crude proteins were assessed in animals at 111–120 kg of live body weight (loin, 23.63%), while the lowest average values were found in animals with live body weight of 121 kg and more (neck, 18.82%). In this regard, Correa *et al*. [19] obtained results for crude proteins content in the range from 23.30 to 23.80%, when the highest values were in the group of animals with the average live weight of 115 kg. Also, Ševčíková *et al*. [21] mentions 23.11 and 22.71 % of crude proteins content. In recent years, high consumer demand for low-fat pork has increased the production of lean genotype pigs. The genetic potential for maximum protein accretion and the slope of the linear response phase of protein accretion generally varies in modern commercial pigs [22].

Table 3. Content of proteins (%) in relation to live weight in major meaty parts of pork; $\overline{X}\pm S_d$, $\overline{X}$ – mean value; S_d – standard deviation; $p < 0.01$ – the same small letters; $p < 0.05$ – the same capital letters

Live weight kg	Meaty parts			
	Neck	Loin	Shoulder	Ham
100–110	17.78± 1.05	22.54± 0.79	17.96^{AB}± 0.80	21.01± 1.10
111–120	18.31± 0.68	22.33± 0.59	18.92^{A}± 0.84	20.60± 0.44
121–130	17.43± 1.04	22.43± 0.81	18.55^{B}± 0.54	20.96± 1.41

The Table 4 characterizes contents of water in relation to live weight in major meaty parts of pork. The highest average water value was found in ham (74.04%) and loin (71.87%), in particular, 74.21% in ham and 72.34% in loin in animals at 100-110 kg of live body weight. Contrary to this, the lowest average value was recorded in the neck, in particular, 65.52% in the group with live weight of 121–130 kg. Similar results were obtained by Ševčikova *et al*. [21] in the hybrid combination of Landrace and Yorkshire with an average live weight of 114 kg and interval between 73.58 and 74.27%. In research conducted by Okrouhlá *et al*. [20] with hybrid pigs in the Czech Republic, the highest average water value was found in the shoulder (76.27%) in the group up to 100 kg live weight, while the lowest average value was achieved for the neck (67.15%) in the group with live weight of 120 kg and more.

Table 4. Content of water (%) in relation to live weight in major meaty parts of pork; $\overline{X}\pm S_d$, $\overline{X}$ – mean value; S_d – standard deviation

Live weight kg	Meaty parts			
	Neck	Loin	Shoulder	Ham
100–110	66.76±2.42	72.34±1.34	67.66±3.32	74.21±1.00
111–120	67.22±1.17	71.58±1.85	69.34±1.28	74.43±1.08
121–130	65.52±2.35	71.57±1.22	70.51±2.70	73.36±1.26

The recorded percentage of intramuscular fat was different for each group and for each part of meat (Table 5). The highest intramuscular fat presence was in the group with live weight 100–110 and 121–130 kg (neck, 13.16 and 14.11%) and the lowest intramuscular fat content was in the group with live weight 100–110 and 111–120 kg (ham, 2.41 and 2.47%). The group with live weight of 100–110 kg showed average values of intramuscular fat content in the interval between 13.16 (neck) and 2.41% (ham). Animals at 111–120 kg of live weight had the average intramuscular fat values in the interval between 11.49 (neck) and 2.47% (ham). In the group of 121–130 kg of live weight, the average values fluctuated between 14.11 (neck) and 2.72% (ham).

Table 5. Content of intramuscular fat (%) in relation to live weight in major meaty parts of pork; $\overline{X} \pm S_d$*,* $\overline{X}$ *– mean value;* S_d *– standard deviation*

Live weight kg	Meaty parts			
	Neck	Loin	Shoulder	Ham
100–110	13.16±3.28	2.78±1.07	12.05±3.75	2.41±0.77
111–120	11.49±1.59	3.60±1.58	9.28±1.98	2.47±1.14
121–130	14.11±3.18	3.02±1.43	7.99±2.90	2.72±2.35

One of the main factors affecting sensory meat quality is intramuscular fat content, which has been positively correlated with meat tenderness, juiciness, and taste. A high level of intramuscular fat, termed marbling fat in muscle, has been associated with improved eating quality of the meat [24]. Intramuscular fat content values (2.52–3.34%) met the requirements for good sensory characteristics of meat defined by Fernandez *et al*. [23]. Matoušek *et al*. [25] found out 2.39% share of intramuscular fat in hybrid population of pigs. As stated by Mikule *et al*. [26], the average content of intramuscular fat in the monitored pigs breed in the Czech Republic was between 1.02–1.94%. The optimum range of intramuscular fat content for meat acceptability was suggested to be 2 to 3%. However, in recent decades pigs have been selected for high lean content by opting for decreased back fat thickness and high growth capacity. Because back fat thickness and intramuscular fat content are genetically correlated (approximately 0.37), intramuscular fat content of pig meat has decreased below the suggested level for meat acceptability.

The challenge is to raise the intramuscular fat content of muscle to a level that will satisfy the nutrition needs of consumers but at the same time to produce pork with minimal visible fat, which is a deterrent to health-conscious consumers. The most effective procedure for manipulating the fat content and fatty acid composition of pigs is through strategic nutrition [22].

The content of mineral substances as listed in the Table 6 showed different interval values. The highest values (2.98%, loin) were shown in the group with the live weight of 100–110 kg, and the lowest (2.31%, neck) in the group with live weight of 121–130 kg.

The average pH values are presented in the Table 7. The highest average pH values were assessed in the group with 100–110 kg of live weight (neck, 5.77) while the lowest average values were found in the group with live weight 121–130 kg (ham, 5.33). The incidence of pale, soft, and exudative meat is one of the main quality problems in pork. The appearance of this type of meat can be monitored by measuring pH, instrumental colour and exudates loss, as pale, soft, and exudative meat is characterized by its intense paleness, low pH, low consistency and intense exudation. Occurrence of dark, firm and dry meat can be defined according to the ultimate pH values. Forrest [27] reported that ultimate pH value of 6.2 and higher was an indicator of dark, firm and dry meat. Based on the ultimate pH values, all three groups in our investigation exhibited normal values with significant differences between groups in major parts of meaty pork (neck and shoulder, $p < 0.05$; ham, $p < 0.01$).

Table 6. Content of mineral substances (%) in relation to live weight in major meaty parts of pork; $\overline{X} \pm S_d$*,* $\overline{X}$ *– mean value;* S_d *– standard deviation;* $p < 0.01$ *– the same small letters;* $p < 0.05$ *– the same capital letters*

Live weight kg	Meaty parts			
	Neck	Loin	Shoulder	Ham
100–110	$2.31^{Aa}\pm$ 0.17	$2.34^{a}\pm$ 0.18	$2.33^{a}\pm$ 0.17	$2.37^{a}\pm$ 0.18
111–120	$2.48^{Ab}\pm$ 0.10	$2.49^{b}\pm$ 0.11	$2.47^{b}\pm$ 0.10	$2.50^{b}\pm$ 0.13
121–130	$2.94^{ab}\pm$ 0.02	$2.98^{ab}\pm$ 0.01	$2.95^{ab}\pm$ 0.05	$2.97^{ab}\pm$ 0.01

Table 7. Values of pH in relation to live weight in major meaty parts of pork; $\overline{X} \pm S_d$*,* $\overline{X}$ *– mean value;* S_d *– standard deviation;* $p < 0.01$ *– the same small letters;* $p < 0.05$ *– the same capital letters*

Live weight kg	Meaty parts			
	Neck	Loin	Shoulder	Ham
100–110	$5.77^{AB}\pm$ 0.21	5.34± 0.06	$5.65^{A}\pm$ 0.20	$5.35^{a}\pm$ 0.07
111–120	$5.51^{A}\pm$ 0.18	5.47± 0.26	5.55± 0.14	$5.49^{ab}\pm$ 0.10
121–130	$5.50^{B}\pm$ 0.15	5.38± 0.06	$5.45^{A}\pm$ 0.08	$5.33^{b}\pm$ 0.04

Statistical evaluation showed that the differences between the groups were highly significant ($p < 0.05$; $p < 0.01$) for the values of proteins content, mineral substance and pH values, while for the contents of water and intramuscular fat there were no differences between the groups. These results are in disagreement with most studies which showed no variation of protein content with slaughter weight [9,12]. Previous studies indicated some or no effect of slaughter weight on meat quality traits [9,10,12].

CONCLUSIONS

On the basis of the obtained results of measuring it can be documented that slaughter weight did not influence carcass composition of pigs of different body weight. The largest part of the carcass is ham, with a share of about 31% in all pigs. After ham, the front third (neck, blade, shank) makes up the largest share of the carcass, about 26%, followed by ribs with bacon, about 16%, and loin, about 15%, in all groups of fatlings. The highest content of proteins was found in the part of ham in animals at 100–110 kg of live weight (21%), while the lowest content was in the part of neck (17.43%) in animals at 121–130 kg of live weight. The highest/lowest values of water content were in the group of 111–120 kg (shoulder, 74.43%) /121–130 kg of live weight (neck, 65.52%). The highest content of intramuscular fat was found in the part of neck in animals at 121-130 kg of live weight (14.11%), while the lowest content was in the part of ham (2.41%) in animals at 100–110 kg of live weight. The highest/lowest values of mineral substances were obtained in loin/ /neck in the group of animals with live weight of 121– –130 kg and 100–110 kg, respectively. Values of pH ranged from 5.33 to 5.77. Differences between the groups for the values of protein content, mineral substance and pH values were highly significant, while for the contents of water and intramuscular fat there were no differences between the groups. This research has practical application as a starting point in development of pig production and meat processing industry, by providing an optimal body weight of individual genotypes of pigs, the aim of which is to obtain better quality of the final product – fattened pig (fatlings) with optimal organoleptic, nutritional, physical, chemical and technological properties.

Acknowledgments

Research was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, project TR 31032. Also, these results are part of the project No. 114-451-3464/2014 which is financially supported by the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina, Republic of Serbia.

REFERENCES

[1] B. James, R.D. Goodband, J.A. Unruh, M.D. Tokach, J.L. Nelssen, S.S. Dritz, P.R. O'Quinn, B.S. Andrews, Effect off creatine monohydrate on finishing pig growth performance, carcass characteristics and meat quality, Anim. Feed Sci. Techol. **96** (2002) 135–145.

[2] L.I. Chiba, D.L. Kuhlers, L.T. Frobish, S.B. Jungst, E.J. Huff-Lonergan, S.M. Lonergan, K.A. Cummins, Effect of dietary restrictions on growth performance and carcass quality of pigs selected for lean growth efficiency, Livestock Prod. Sci. **74** (2002) 93–102.

[3] M.A. Latorre, R. Lázaro, M.I. Gracia, M. Nieto, G.G. Mateos, Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 117 kg body weight, Meat Sci. **65** (2003) 1369–1377.

[4] J. Gonzalez, J. Soler, M. Gispert, X. Puigvert, J. Tibau, Chemical composition of pig carcasses, Effects of body weight, sex and genotype, ITEA, Extra. **22** (2001) 661– –663.

[5] J. Tibau, J. Gonzalez, J. Soler, M. Gisper, R. Lizardo, J. Mourot, Influence of pig slaughter weight (range 25 to 140 kg) on the chemical composition of the carcass: effect of genotype and sex, J. Res. Porcine. **34** (2002) 121–127.

[6] A. Fortin, Carcass composition of Yorkshire barrows and gilts slaughtered between 85 and 112 kg body weight, Can. J. Anim. Sci. **2** (1982) 69–76.

[7] R. Bečkova, Vliv různe kombinace plemen na podil intramuskularniho tuku u finalnich hybridů. Sbornik z mezinarodni konference „Aktualni problemy šlechtěni, zdravi, růstu a produkce prasat", Č. Budějovice (1996) 36–38 (in Czech).

[8] V. Mikule, Vliv intenzity šlechtěni různych populaci prasat na obsah intramuskularniho tuku. DDP, Brno (2005) 4–17 (in Czech).

[9] M. Ellis, T.M. Bertol, Effects of slaughter weight on pork and fat quality, Proc. 2nd Intern. virtual conf. on pork quality, Concordia, 2001, pp. 213–224.

[10] J.R. Piao, J.Z. Tian, B.G. Kim, Y.I. Choi, Y.Y. Kim, I.K. Han, Effects of sex and market weight on growth performance carcass characteristics and pork quality of market hogs, J. Anim. Sci. **10** (2004)1452–1458.

[11] A. Hugo, G. Osthoff, P.J. Jooste, Effect of slaughter weight on the intramuscular fat composition of pigs, in Proc. of 45th Intern. Cong. of Meat Sci. and Techol. Yokohama, 1999, pp. 496–497.

[12] M.A. Latorre, R. Lázaro, D.G. Valencia, P. Medel, G.G. Mateos, The effects of sex and slaughter weight on the growth performance, carcass traits, and meat quality characteristics of heavy pigs, J. Anim. Sci. **82** (2004) 526– –533.

[13] ISO 1443 (1973). Meat and meat products. Determination of total fat content. International Organisation for Standardisation. Switzerland.

[14] ISO 937 (1978). Meat and meat products. Determination of nitrogen content (reference method). International Organisation for Standardisation, Switzerland.

[15] ISO 2917 (1999). Meat and meat products. Measurement of pH (Reference method). International Organisation for Standardisation, Switzerland.

[16] V. Vidović, D. Lukač, Lj. Štrbac, D. Punoš, V. Višnjić, M. Stupar, M. Dokmanović, Uticaj kriterijuma selekcije u svinjarstvu i kvalitet svinjskih polutki, Vet. glasnik. **67** (2012) 201–214 (in Serbian).

[17] D. Lukač, V. Vidović, Lj. Štrbac, D. Punoš, M. Stupar, The influence of breed and sex on tissue distribution in pig carcasses, in Proc. of 6th Cen. Europ. Cong. Food, Novi Sad, 2012, pp. 1670–1674.

[18] R. Ukmar, Z. Đurkin, Z.Maltar, G. Kralik, A. Petričević, G. Kušec, Mesnatost i sastav klanički obrađenih trupova svinja u Hrvatskoj, Meso **6** (2008) 422–428 (in Croatian).

[19] J.A. Correa, L. Faucitano, J.P. Laforest, J. Rivest, M. Marcoux, C. Gariepy, Effects of slaughter weight on car-

cass composition and meat quality in pigs of two different growth rates. Meat Sci. **72** (2006) 91–99.

[20] M. Okrouhlá, R. Stupka, J. Čítek, M. Šprysl, E. Kluzáková, M. Trnka, Effect of the slaughter weigh on basic chemical composition of pork, Res. Pig Breeding **1** (2007) 1–3.

[21] S. Ševčikova, M. Koucky, J. Laštovkova, Meat performance and quality in different genotypes of F1 generation gilts, Czech J. Anim. Sci. **47** (2002) 395–400.

[22] D. Lukač, V. Vidović, Lj. Štrbac, D. Punoš, V. Višnjić, M. Stupar, M. Dokmanović, Fenotipska i genetska analiza svojstava kvaliteta polutki u svinja, Vet. glasnik **64** (2013) 215–226 (in Serbian).

[23] J.D. Wood, G.R. Nute, R.I. Richardson, F.M. Whittington, O. Southwood, G. Plastow, R. Mansbridge, N. da Costa, K.C. Chang, Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Sci. **67** (2004) 651–667.

[24] X. Fernandez, G. Monin, A. Talmant, J. Mourot, B. Lebret, Influence of intramuscular fat content on the quality of pig meat – 2. Consumer acceptability of m. *Longissimus lumborum*, Meat Sci. **53** (1999) 67–72.

[25] V. Matoušek, N. Kernerova, J. Vaclavovsky, A. Vejčik, Analyza kvality masa u hybridni populace prasat. Živ.vyr. **42** (1997) 511–515.

[26] V. Mikule, M. Čechova, L. Sladek, An Influence of improving on higher meatness on a content of intramuscular fat in pork, Acta Uni. Agric. Silvic. Mendelianea Brunensis **3** (2002) 135–140.

[27] J.C. Forrest, Line speed implementation of various pork quality measures, in Proc. of NSIF Conf. Ann. Meet., Vol. 23, East Lansing Marriot, MI, 1998.

IZVOD

OSNOVNI HEMIJSKI SASTAV MESA I KVALITET TRUPA HIBRIDNIH TOVLJENIKA RAZLIČITE TELESNE MASE NA KLANJU

Dragomir R. Lukač[1], Vitomir S. Vidović[1], Aleksandar Lj. Stoisavljević[2], Nikola M. Puvača[1], Natalija R. Džinić[3], Vladimir M. Tomović[3]

[1]*Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za stočarstvo, Novi Sad, Srbija*
[2]*PTK Panonija a.d., BačkaToplola, Serbia*
[3]*Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad*

(Naučni rad)

Meso predstavlja veoma bogat i jedinstven izvor hranljivih materija. Posebna važnost mesa se bazira na sadržaju proteina. Optimalna telesna masa prilikom klanja utiče na osnovni hemijski sastav mesa. Tovljenici pre klanja su podeljeni u tri grupe. Najveći sadržaj proteina i vode se nalazio u butu i plećki u grupama od 100 do 110 kg i 111 do 120 kg, dok je najmanji sadržaj proteina i vode bio u vratu kod svinja telesne mase od 121–130 kg. Najveći sadržaj intremuskularne masti i mineralnih materija je zabeležen u grupi svinjas atelesnom masom od 121 do 130 kg (vratu i kareo), dok je najmanji sadržaj zabeležen u grupi svinja telesne mase od 100 do 110 kg (but). Kiselost mesa (pH) se kretala u normalnim granicama (5,33––5,77). Razlike između grupa u sadržaju proteina, mineralnih materija kao i pH mesa su bile visoko statistički značajne ($p < 0,05$; $p < 0,01$), dok razlike u sadržaju vode i intramuskularne masti nisu zabeležene između posmatranih grupa ($p > 0,05$).

Ključne reči: Tov • Svinje • Klanična masa • Hemijski sastav • Meso

Constructed wetlands in the treatment of agro-industrial wastewater: A review

Mar-Yam Sultana[1], Christos S. Akratos[1], Dimitrios V. Vayenas[2,3], Stavros Pavlou[2,3]

[1]Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece
[2]Institute of Chemical Engineering Sciences, FORTH, Patras, Greece
[3]Department of Chemical Engineering, University of Patras, Patras, Greece

REVIEW PAPER

UDC 504.5(497.1)"1999":
546.791623.454.8:631.4(497.1)

Hem. Ind. **69** (2) 127–142 (2015)

doi: 10.2298/HEMIND150121018S

Abstract

Due to their simplicity and low operation cost, constructed wetlands are becoming more prevalent in wastewater treatment all over the world. Their range of applications is no longer limited to municipal wastewater, but has expanded to the treatment of heavily polluted wastewaters such as agro-industrial effluents. This paper provides a comprehensive literature review of the application of constructed wetlands in treating a variety of agro-industrial wastewaters, and discusses pollutant surface loads and the role of constructed wetland type, prior-treatment stages and plant species in pollutant removal efficiency. Results indicate that constructed wetlands can tolerate high pollutant loads and toxic substances without losing their removal ability, thus these systems are very effective bio-reactors even in hostile environments. Additionally, the review outlines issues that could improve pollutant treatment efficiency and proposes design and operation suggestions such as suitable vegetation, porous media and constructed wetland plain view. Finally, a decision tree for designing constructed wetlands treating agro-industrial wastewaters provides an initial design tool for scientists and engineers.

Keywords: constructed wetlands, agro-industrial wastewater, review, decision tree, design.

Available online at the Journal website: http://www.ache.org.rs/HI/

Constructed wetlands (CWs) are a low-cost technology that has been used to treat various types of wastewaters for nearly twenty years [1–2]. They are an attractive treatment option because they use solar energy, are simple to construct and operate, have low maintenance cost and are inexpensive and sustainable compared to conventional treatment methods [3]. The water quality improvement observed depends on the wetland design, microbial community, and the different plant species involved [4].

Constructed wetlands are artificial systems that have been designed to operate as natural wetland ecosystems to improve wastewater treatment efficiency. These systems generally fall into two general categories: a) subsurface flow system wetlands, and b) free water surface systems. In recent years, constructed wetland technology has been based on three basic types of CWs: a) free water surface (FWS) wetlands, b) horizontal subsurface flow (HSF) wetlands and c) Vertical flow (VF) wetlands.

Lately, different CW types have been paired into hybrid treatment systems. Hybrid CW systems have also been used extensively in wastewater treatment. These hybrid systems mainly consist of different HSF and VF stages and are used to achieve high organic matter removal efficiency and high nitrification rates from the VF CWs, and high denitrification rates from the HSF CWs.

CWs are mainly used to treat domestic and municipal wastewaters, but more recent applications of CWs include treatment of other types of wastewater, such as industrial, agro-industrial and agricultural wastewaters, various runoff waters and landfill leachate [5].

Agro-industry includes post-harvest activities involved in the transformation, preservation and preparation of agricultural products for intermediary states or final consumption. Agro-industrial wastewaters are usually characterized by their high organic load and their quantity and quality variations over a year [6].

Although previous papers have partially reviewed CWs treating agro-industrial wastewater, they are either dated [7] or agro-industrial wastewater is only a limited part of the paper and not thoroughly discussed [8]. This paper provides a comprehensive literature review of the application of CWs in treating a variety of agro-industrial (*i.e.*, dairy, animal farm, winery, trout farm, sugar production and olive mill) wastewaters, as CW application is no longer limited to municipal wastewaters. To evaluate comprehensively the ability of CWs to treat these wastewaters, certain parameters are examined and discussed thoroughly. These parameters include CW type, prior-treatment stages, and plant

---

Correspondence C.S. Akratos, Department of Environmental and Natural Resources Management, University of Patras, G. Seferi Str. 2, GR-30100 Agrinio, Greece.
E-mail: cakratos@upatras.gr
Paper received: 21 January, 2015
Paper accepted: 16 March, 2015

species. Other significant parameters such as CW surface area and pollutant surface loads for specific toxic pollutants (*e.g.*, phenols) are discussed in detail. Furthermore, the review provides design and operation suggestions including issues such as vegetation, porous media, CW plain view and others, by introducing an innovative decision tree for designing CWs treating agro-industrial wastewaters.

PRIOR-TREATMENT STAGES

CWs were mainly used as a polishing treatment method for agro-industrial wastewater treatment, thus several prior-treatment technologies are employed (Table 1). These technologies aim to remove suspended solids and reduce organic matter loads. Suspended solid removal is essential before the wastewaters enter the CW stage, because high suspended solid concentrations can cause clogging of the porous media. The necessity of pre-treatment stage was also outlined in previous reviews, where it was mentioned that CWs treatment facilities were coupled mainly by settling basins, lagoons, septic tanks in order to mainly remove SS [7]. Therefore, in the majority of experiments/applications referred in the literature, the pre-treatment stages include either simple settling basins [3,9–22] or stabilization lagoons, which can achieve high removal rates of suspended solids and organic matter [4,23–27].

According to the specifications of each wastewater, prior-treatment stages can also include other technologies. Winery wastewater treatment usually includes a sludge digestion stage to remove suspended solids and significantly reduce organic loads [28]. Animal farm wastewaters that contain high organic and nitrogen

Table 1. Prior-treatment stages used in CWs treating agro-industrial wastewater

Prior-treatment stage	Wastewater Type	Reference
	FWS	
Stabilization lagoon	Animal farm	[1,35–39]
	Dairy	[40]
Dilution	Animal farm	[41]
	OMW	[42]
Settling tanks	Dairy	[3,12,16]
	Animal farm	[19]
	Hybrid	
Settling tanks	Dairy	[15]
Stabilization lagoons	Animal farm	[25]
	Vinegar	[26]
Septic tank	Dairy, swine and potato starch	[31]
Sludge digester	Winery	[28]
	HSF	
Biological treatment	Dairy- Cheese whey	[2,43,44]
	Animal	[30]
Stabilization lagoons	Dairy	[4,23,27]
	Animal farm	[24]
Settling tank	Dairy	[9–11,13,17,20,21]
	Animal farm	[18]
	Winery	[14]
	Trout farm	[22]
Solid separation, anaerobic digestion and aerobic oxidation	Animal	[29]
Sludge digestion	Animal	[45,46]
	Winery	[47]
Coagulation	OMW	[33,48]
Dilution	Sugarcane	[49]
	VF	
Dilution	OMW and swine	[50]
	Cheese whey	[51]
Biological filter	OMW	[34]
Electrochemical oxidation	OMW	[32]

loads are mainly treated with various biological treatment methods to reduce both organic and nitrogen loads [29–31]. The main treatment stages for OMW involve advanced treatment methods including coagulation [32], electrochemical oxidation [33], and biological trickling filters [34] to lower toxic phenol concentrations.

Several experiments/applications on agro-industrial wastewater treatment using CWs lack a main treatment stage [41,42,49-52]. In these experiments/applications the wastewaters were diluted with tap water before being introduced into the CW system.

Although the majority of CWs experiments/applications on agro-industrial wastewaters refer to prior-treatment stages and they do not discuss the specific aspects of these stages. Thus, limited information is available concerning the significance and effectiveness of prior-treatment stages on CW performance. Nevertheless, some critical information can be drawn. Specifically, although physicochemical methods (e.g. coagulation) can successfully remove TSS, they use chemical compounds (*e.g.*, calcium hydroxide, lime putty and hydraulic lime). This leads to the increase of pH values to above 8, which could be toxic to CW vegetation [32]. On the other hand, the electrochemical oxidation as a prior-treatment stage to CWs treating OMW results in lower pollutant removal efficiencies than when it is used as a polishing stage after CW treatment [33]. Therefore it seems that a biological treatment method should be applied as a prior-treatment stage to CWs [33,34]. As agro-industrial wastewaters are characterized by high pollutant loads, aerobic suspended growth systems would probably not achieve satisfactory removal efficiencies. In contrast, aerobic attached growth systems (*e.g.*, trickling filters) or anaerobic systems could operate under these high pollutant loads and could achieve high removal rates.

VEGETATION

One of the main issues in CW treatment systems is to identify the precise role of the plant vegetation in pollutant removal and define their toxicity boundaries. Several plant species have been used in CWs treating agro-industrial wastewater treatment (Table 2). The exact contribution of plants in nutrient removal is a controversial issue as almost all related studies give different removal efficiencies. Gottschall *et al.* [36] report that nutrient removal due to plant uptake was significantly lower in their study compared to previous studies [53,54] which reported that plant uptake is responsible for 27–66% of nitrogen removal and 47–65% of phosphorus removal. In addition, Newman *et al.* [11] report that only 3% of nitrogen removal should be attributed to plant uptake. Mantovi *et al.* [13] also attribute most nutrient removal to biofilm biochemical oxidation and plant uptake. Tanner *et al.* [23] reported that planted wetlands showed greater removal efficiencies of N and P from dairy farm wastewaters than unplanted wetlands. They recorded higher TP removal in the summer months due to higher plant biomass growth and temperatures. The unplanted wetland proved to be less efficient at removing both N and P with higher loading rates. Percentage removal of NH_4^+–N increased with retention time in the planted HSF CW, whereas the unplanted wetland showed lower performance. Plant rhizosphere aeration may stimulate aerobic decomposition processes by increasing nitrification and subsequent gaseous losses of N through denitrification [55,56], and by decreasing the relative levels of dissimilatory nitrate reduction to ammonium [57].

Table 2. Plant species used in CWs treating agro-industrial wastewater

Plant species	Influent concentration, mg/L			CW Type	Wastewater type	Reference
	COD	TN	P			
Acorus calamus	1700	360	–	HSF	Animal farm	[46]
Alisma plantago-aquatica	1700	360	–	FWS	Dairy	[16]
				HSF	Animal farm	[46]
Butomus umbellatus	–	–	–	Hybrid	Dairy	[15]
Cacomantis flabelliformis	1034	448	–	FWS	Animal farm	[19]
Carex spp.	682–1700	11–360	2.3–10.4	FWS	Dairy	[16]
				HSF	Dairy	[10]
				HSF	Dairy	[59]
				HSF	Animal farm	[46]
				Hybrid	Vinegar	[26]
Ceratophyllum demersum	721–4045	14.7–65.2	1.9–4.9	FWS	Winery	[14]
Cucurbita maxima	–	–	–	Hybrid	Dairy	[15]
Eichhornia crassipes	1160	200	40	HSF	Animal farm	[29]
Eleocharis obtusa	–	–	–	HSF	Dairy	[9]

Table 2. Continued

Plant species	Influent concentration, mg/L			CW Type	Wastewater type	Reference
	COD	TN	P			
Elodea canadensis	721–4045	14.7–65.2	1.9–4.9	FWS	Winery	[14]
Filipendula ulmaria	1700	360	–	HSF	Animal farm	[46]
Glyceria spp.	682–1500	97	10.4	FWS	Dairy	[16]
				HSF	Dairy	[10]
				HSF	Dairy	[59]
				HSF	Dairy	[60]
Holcus lanatus	–	–	–	HSF	Dairy	[60]
Iris pseudacorus	1700	360	–	FWS	Dairy	[16]
				HSF	Animal farm	[46]
Juncus spp.	–	20–120	20–50	FWS	Animal farm	[1]
				FWS	Animal farm	[61]
				HSF	Animal farm	[62]
Lemna spp.	254	29	17	FWS	Dairy	[3]
				HSF	Dairy	[27]
Litaneutria minor	1900	164	53	FWS	Dairy	[12]
				HSF	Dairy	[9]
Lythrum salicaria	1700	360	–	HSF	Dairy	[9]
				HSF	Animal farm	[46]
Mentha aquatica	1700	360	–	HSF	Animal farm	[46]
Nymphea rustica	721–4045	14.7–65.2	1.9–4.9	FWS	Winery	[14]
Nuphar lutea	–	20–120	20–50	HSF	Animal farm	[62]
Phalaris arundinaceae	6.79–1500	6.16	0.34	FWS	Dairy	[16]
				HSF	Dairy	[59]
				HSF	Trout farm	[26]
Phormium tenax	–	–	–	HSF	Dairy	[60]
Phragmites spp.	6.79–14000	6.16–506	0.34–95	FWS	Dairy	[16]
				FWS	Dairy	[12]
				FWS	Dairy	[40]
				Hybrid	Dairy	[15]
				Hybrid	Dairy	[63]
				HSF	Dairy	[64]
				HSF	Dairy	[17]
				HSF	Dairy	[4]
				HSF	Dairy	[10]
				HSF	Dairy	[13]
				HSF	Dairy	[9]
				HSF	Dairy	[11]
				HSF	Cheese whey	[44]
				HSF	Animal farm	[24]
				HSF	Animal farm	[45]
				HSF	Animal farm	[46]
				HSF	Animal farm	[30]
				VF	Animal farm	[48]
				VF	Animal farm	[65]
				HSF	OMW	[48]
				VF	OMW	[34]
				FWS	OMW	[42]
				FWS, HSF, VF	Winery	[14]

Table 2. Continued

Plant species	Influent concentration, mg/L			CW Type	Wastewater type	Reference
	COD	TN	P			
Phragmites spp.	6.79–14000	6.16–506	0.34–95	Hybrid	Vinegar	[26]
				HSF	Trout farm	[26]
				VF	OMW and swine	[50]
				Hybrid	Winery	[28]
				Hybrid	Dairy, swine and potato starch	[31]
				Hybrid	Winery	[47]
Pontederia spp.	254–1200	20–120	22–50	HSF	Dairy	[27]
				HSF	Animal farm	[62]
				HSF	Sugarcane	[49]
Schoenoplectus americanus	445–796	66–171	56–71	FWS	Animal farm	[39]
				FWS	Animal farm	[61]
				FWS	Animal farm	[37]
				FWS	Animal farm	[66]
				FWS	Animal farm	[38]
				Hybrid	Animal farm	[25]
Scirpus spp.	285–2240	20–907	20–53	FWS	Dairy	[12]
				FWS	Dairy	[40]
				HSF	Dairy	[4]
				HSF	Dairy	[23]
				FWS	Animal farm	[1]
				FWS	Animal farm	[61]
				HSF	Animal farm	[62]
				HSF	Animal farm	[18]
				HSF	Animal farm	[24]
				HSF	Animal farm	[46]
Solanum americanum	–	–	–	FWS	Animal farm	[1]
				FWS	Animal farm	[61]
Sparganium erectum	1700	360	–	FWS	Dairy	[16]
				Hybrid	Dairy	[15]
				Hybrid	Animal farm	[25]
				HSF	Animal farm	[46]
Senecio sylvaticus	–	–	–	Hybrid	Dairy	[15]
Sotalia fluviatilis	1160	200	40	Hybrid	Dairy	[67]
				HSF	Dairy	[21]
Suillus pungens	2700	102	26	HSF	Dairy	[9]
				HSF	Dairy	[11]
Stuckenia pectinata	682	97	10.4	HSF	Dairy	[10]
Typha spp.	150–4045	14.7–360	10.4–71	FWS	Dairy	[16]
				FWS	Dairy	[3]
				FWS	Dairy	[12]
				FWS	Dairy	[40]
				Hybrid	Dairy	[15]
				HSF	Dairy	[2]
				HSF	Dairy	[10]
				HSF	Dairy	[59]
				HSF	Dairy	[9]
				HSF	Dairy	[11]
				HSF	Dairy	[20]

Table 2. Continued

Plant species	Influent concentration, mg/L			CW Type	Wastewater type	Reference
	COD	TN	P			
Typha spp.	150–4045	14.7–360	10.4–71	FWS	Animal farm	[36]
				FWS	Animal farm	[1]
				FWS	Animal farm	[39]
				FWS	Animal farm	[61]
				FWS	Animal farm	[37]
				FWS	Animal farm	[66]
				FWS	Animal farm	[38]
				Hybrid	Animal farm	[25]
				HSF	Animal farm	[62]
				HSF	Animal farm	[24]
				HSF	Animal farm	[46]
				HSF	Animal farm	[30]
				VF	OMW	[33]
				FWS, HSF, VF	Winery	[14]
				VF	Cheese whey	[52]
Urtica dioica				Hybrid	Dairy	[15]

The selection of appropriate vegetation in CWs treating agro-industrial wastewater is an important issue as toxic effects caused by high organic and nutrient loads can occur. *Typha* spp. and *Phragmites* spp. are most commonly used for various agro-industrial wastewaters (*i.e.*, dairy, animal farm, winery, vinegar, trout farm, potato starch and OMW). Apart from *Typha* and *Phragmites* thirty-one other species have been used in CWs treating agro-industrial wastewater. Of these 31 species, *Carex* spp. and *Scirpus* spp. are the most frequently used in CWs treating dairy and animal farm wastewaters. Although a significant number of plant species have been used in CWs treating agro-industrial wastewaters no significant observations have been made concerning the effect of the different species on pollutant removal efficiencies. On the contrary, the comparative studies with different species showed that all were able to grow in CWs treating dairy wastewater without showing toxicity [15] and did not show any significant differences on organic matter removal [58]. Cronk [7] also suggested that CWs vegetation should be native and tolerant to extreme pollutant loads, while they should also have rapid growth and significant nutrient uptake.

Phragmites

Phragmites spp. is recognized as the most popular plant used in CW applications [68]. Its extensive use is due to: a) its high biomass productivity (up to 9,890 g dry mass/m^2 per year), b) its ability to grow in fresh and saline waters and c) its natural widespread distribution [68]. *Phragmites* spp. is also extensively used in CW applications for agro-industrial wastewater treatment. *Phragmites* spp. appears to be extremely tolerant to high concentrations of organic matter and nutrients and does not show toxic effects to COD, TKN and TP concentrations of up to 14000, 506 and 95 mg/L, respectively (Table 2).

Typha

Along with *Phragmites* spp., *Typha* spp. is the most commonly used plant in CWs, and they share common characteristics of high biomass productivity, tolerance to brackish waters and a widespread distribution [68]. *Typha* spp. is used in all CW types to treat a variety of agro-industrial wastewaters (i.e. dairy, cheese whey, animal farm, OMW and winery). *Typha* spp. is tolerant to high organic matter and nutrient concentrations, as it has been used in experiments/applications in which COD, TKN and TP concentrations were up to 4000, 360 and 71 mg/L, respectively (Table 2). These concentrations are rather high and prove that *Typha* spp. can be used successfully in CWs treating high strength wastewaters. On the contrary, Ghosh and Gopal [2] examined plant tolerance to dairy wastewater and found that young *Typha* plants yellowed when wastewater with high EC values was applied to the CWs. Ghosh and Gopal [2] also mentioned that plant density and height were maximum near the CW's inlet and attributed this to the higher nutrient concentrations present in these areas which promote plant growth. Concerning nutrient uptake from *Typha* spp., Gottschall *et al.* [36] found that N was absorbed in *Typha* spp. biomass mainly as NH_4^+ and not as NO_3^-, while P uptake was significant lower.

FWS CWS TREATING AGRO-INDUSTRIAL WASTEWATERS

FWS CWs have been extensively used to treat a variety of agro-industrial wastewaters (*i.e.*, dairy, animal farm and OMW) (Table 3). These applications range from pilot-scale experiments to full-scale facilities as their areas vary from 120.6 to 4000 m^2 (Table 3). FWS CWs have been operated with various pollutant surface loads ranging from 1.9 to 259.4 g/m^2 per day for organic matter, from 0.4 to 77 g/m^2 per day for TKN, from 0.05 to 12.7 g/m^2 per day for phosphorus, and from 2.55 to 949 g/m^2 per day for TSS. Removal efficiencies in these CWs show great variations, ranging from 3 to 98% for organic matter, from 26 to 96% for TKN, from 8 to 92% for TP, and from 26 to 99% for TSS, depending on the HRT applied and the metrological conditions. In most cases the high removal efficiencies were achieved when pollutant surface loads were low (Table 3).

A common characteristic of all FWS CWs treating agro-industrial wastewater is that influent COD concentration does not exceed 2000 mg/L, as at higher concentrations FWS CWs cannot efficiently remove pollutants due to the anoxic or anaerobic conditions created in the water column, which reduces the amount of oxygen available for microbial organic matter oxidization. According to Schaafsma *et al.* [12], organic matter and nutrient removal efficiencies are also significant, but in some cases, nitrate and nitrite concentrations are increased in the effluent. To enhance denitrification, Schaafsma *et al.* [12] suggested increasing plant density and partially recirculating wastewater. Concerning nitrogen removal, Gottschall *et al.* [36] showed that TKN removal was lower than NH_4^+ removal. The wetland was NH_4^+-dominated and showed a greater uptake of NH_4^+ than NO_3^-. The only attempt to treat OMW using FWS [42] showed significant removal efficiency for organic matter (86%). However, the organic surface load applied was the lowest for OMW reported in the literature (5–15 g/m^2 per day).

HRTs applied on FWS CWs range from 4 to 120 days and significantly affect wetland performance. When low HRTs were used (4 to 15 days) removal efficiencies were low and ranged from 40 to 60% [37,38,40]. On the other hand, high removal efficiencies were achieved for HRTs exceeding 60 days [3,36,61].

HSF CWS TREATING AGRO-INDUSTRIAL WASTEWATERS

HSF CW systems appear to be favoured for agro-industrial wastewater treatment compared to FWS CWs, as they have been used to treat a greater variety of wastewaters (*i.e.*, dairy, animal farm, OMW, winery, sugarcane and trout farm). They have been used at several scales from laboratory experiments to full-scale applications with surfaces areas ranging from 0.25 to 7600 m^2 (Table 4). HSF CWs operated under similar pollutant surface loads with FWS CWs varying from 0.17 to 376 g/m^2 per day for organic matter, 0.007 to 2.7 g/m^2 per day for TKN, 0.004 to 4.7 g/m^2 per day for phosphorus, and from 0.2 to 62.4 g/m^2 per day for TSS. Removal efficiencies in HSF CWs are similar to those recorded in FWS CWs as they range from 28 to 99% for organic matter, 10 to 99% for TKN, 2 to 99% for TP, and from 76 to 99% for TSS, depending on the applied HRT and the meteorological conditions (Table 4).

Although pollutant surface load and removal efficiencies of HSF CWs do not differ greatly from those in FWS CWs, HRTs applied in HSF CWs were lower and ranged from 2 to 60 days. Even with a 2-day HRT, HSF CWs can achieve high organic matter removal efficiencies (up to 90%) [44,49] and this establishes them as far more efficient bio-reactors than FWS CWs.

Table 3. FWS CWs treating agro-industrial wastewater treatment by CWs

Ref.	Wastewater type	CW surface area, m^2	Surface load, g/m^2 per day				Removed surface load, g/m^2 per day			
			C	TKN	TP	TSS	C	TKN	TP	TSS
[1]	Animal farm	120.6	2.74	3.5	–	–	–	–	–	–
[3]	Dairy	100	6.5	0.8	0.12	4.8	6.37	0.64	0.11	4.61
[12]	Dairy	500	–	–	–	–	–	–	–	–
[16]	Dairy	4265	6.84	–	0.05	2.55	6.22	–	0.04	2.52
[36]	Animal farm	327	–	0.95–1.62	0.33–0.34	–	–	0.38–0.65	0.05	–
[37]	Animal farm	220	7.14	1.23	1.1	24.2	4.2	0.7	0.32	15.0
[38]	Animal farm	440	1.9–6.1	0.7–4.0	0.8–1.6	4.4–8.2	0.9–2.7	0.3–1.8	0.16–0.32	1.8–3.3
[39]	Animal farm	440	8.13–87.1	0.4–3.5	–	3425–35109	3.3–34.5	0.2–1.6	–	1370–14044
[40]	Dairy	630	20.36	23.2	–	67.36	0.61	6.0	–	24.2
[42]	OMW		5–15	–	–	–	4.3–12.9	–	–	–
[61]	Animal farm	241.2	10.7–12	3.7–4.4	1–1.6	11.5–18.8	6.4–7.2	2.9–3.5	0.3–0.5	10.6–17.3
[66]	Animal farm	440	–	2.3	1.2	–	–	0.9	0.09	–

Table 4. HSF CWs treating agro-industrial wastewater treatment by CWs

Ref.	Wastewater type	CW surface srea, m^2	Surface load, g/m^2 per day				Removed surface load, g/m^2 per day			
			C	TKN	TP	TSS	C	TKN	TP	TSS
[9]	Dairy	138.6	52.6	1.9	0.514	14.8	14.7	0.5	0.14	6.7
[10]	Dairy	10–15	26.8–45.3	–	–	–	24.1–40.8	–	–	–
[11]	Dairy	138.6 (each cell)	51.3	1.96	0.5	24.6	38.9	0.55	0.23	22.1
[13]	Dairy	72	110.1	5.84	1.15	62.3	101.3	2.83	0.70	56.6
[14]	Winery	127–850	23.6–35.2	0.6–2.7	0.08–0.2	–	22.4–33.4	0.4–1.9	–	–
[17]	Dairy	50–1900	5.7–8.3	0.56–1.97	0.17–1.71	–	4.5–6.6	0.2–0.8	0.12–1.2	–
[18]	Animal farm	18	9.7–47.3	0.7–4.54	–	–	4.85–23.6	0.42–2.7	–	–
[2]	Dairy	1.63	8.6–34.5	1.3–4.9	OP: 0.3–1.1	0.2–0.7	5.2–20.7	0.78–2.94	OP: 0.09–0.33	0.16–0.56
[20]	Dairy	100	17	–	–	–	16.7	–	–	–
[21]	Dairy	892	68.5	–	0.6	16	–	–	–	–
[22]	Trout farm	23.9	9.8	8.9	0.49	–	19.8	1.1	0.27	–
[23]	Dairy	19	0.9–4.1	0.6–2.7	0.2–0.8	1.9–8.5	0.76–3.5	0.4–1.8	0.12–0.48	1.5–6.5
[24]	Animal farm	6000	0.17–0.26	0.15–0.68	–	0.21–1.85	0.12–0.18	0.09–0.48	–	0.07–0.93
[27]	Dairy	398	29.4	3.4	2	8.4	11.2	1.45	0.5	4.9
[29]	Animal farm	31.1	39–137	6.9–26.2	1.5–4.7	30.3–62.4	31.2–110	1.03–3.9	0.7–2.1	29.4–60.5
[30]	Animal farm	0.9	4.2	–	0.2016	6.1	–	–	–	–
[4]	Dairy	600	–	–	–	–	–	–	–	–
[43]	Dairy	20	–	–	–	–	–	–	–	–
[44]	Cheese whey	1.1	19–110	–	–	–	2–109	–	–	–
[45]	Animal farm	0.25	3.2	0.104	0.265	–	2.24	0.08	0.21	–
[46]	Animal farm	4500	0.14	0.03	0.005	–	0.13	0.03	0.005	
[47]	Winery	350	2–49	–	–	–	1.4–34.3	–	–	–
[48]	OMW	0.85	77.03 Phenols: 16.85	1.08	0.42	9.32	53.1 Phenols: 13.2	0.13	0.23	4.6
[49]	Sugarcane	0.9	47.2–94.8	–	–	–	37.8–75.8	–	–	–
[59]	Dairy	7600	1.2	–	–	0.21	1.14	–	–	0.2
[60]	Dairy	260	–	0.7– 2.9	0.004–0.013	–	–	0.4–1.7	0.0004–0.0013	–
[62]	Animal farm	4500	–	0.007	0.004	–	–	0.005	0.001	–
[64]	Dairy	160	–	–	–	–	–	–	–	–

Nevertheless, HRT and pollutant surface loads seem to be important parameters in HSF CWs as, according to Lee *et al*. [29], removal efficiencies are higher when surface loads are low, while Meers *et al*. [45] suggested that by increasing the HRT and the plant root depth zone in HSF CWs, removal efficiencies would increase. HSF CWs appear to be more efficient at treating OMW [32], as removal efficiencies reach 69% for COD, 12% for nitrogen, 55% for phosphorus, 50% for TSS and 79% for phenols. It should be mentioned that these removal efficiencies were achieved with pollutant surface loads higher than those applied to FWS systems, but lower than those applied to VF systems.

HSF CW operation is greatly influenced by HRT, as extremely low HRTs can lead to insufficient treatment, while high HRTs do not improve CW performance and lead only to higher area demand. Ghosh and Gopal [2] state that while organic matter removal increased nearly 3-fold when HRT increased from 1 to 2 days, when HRT was further increased to 3 days organic matter removal increased only by 18%. Sultana et al. [44] also state that HRTs ranging from 2 to 8 days have no significant effect on organic matter removal, while an HRT of 1 day was found to be inadequate for organic matter removal. Almost the same HRT range (2 to 7 days) was also proposed by Tanner *et al*. [23] for successful organic matter removal from dairy wastewater. On the other hand, N and P removal seems to be more affected by HRT, as when HRT increases from 1 to 4 days, N and P removal rates increase up to 4 times [2,23]. Munoz *et al*. [21] suggest that artificial aeration (1.13 m^3/min) leads to clogging elimination. Although clogging is a major problem in CW operation, continuous aeration greatly increases operation cost.

VF AND HYBRID CWS TREATING AGRO-INDUSTRIAL WASTEWATERS

In contrast to FWS and HSF, VF CWs (Table 5) can treat extremely higher pollutant surface loads ranging from 10 to 6589 gr/m^2 day for organic matter, 0.6 to 575 g/m^2 per day for TKN, 0.08 to 20 g/m^2 per day for phosphorus, and 35 to 1836 g/m^2 per day for TSS. Although VF CWs received pollutant surface loads up to 10 times greater than FWs and HSF CWs, they appear to be extremely efficient as removal rates range from 24 to 95% for organic matter, 10 to 99% for TKN, 47 to 95% for TP, and from 21 to 99% for TSS. For some specific agro-industrial wastewaters such as OMW [32–34,51], VF CWs show high removal efficiencies for all pollutants (72–86% for COD, 75% for nitrogen, 88–95% for phosphorus, 79% for phenols), while the pollutant surface loads applied were among the highest reported for all agro-industrial wastewaters. It appears from the literature that VF CWs are more efficient at organic matter and phenol removal, but attention should be paid to the existence of TSS, which can cause porous media to clog and thus damage the CW system. Yalcuk *et al*. [51] attributed VF CW treatment ability to the efficient diffusion of oxygen in CW pores. The higher oxygen concentration in VF CWs results in increased organic matter oxidation and ammonia nitrification. Nevertheless, TN removal in VF CWs is limited due to insufficient denitrification. A viable solution to this problem is to recirculate treated wastewater, which leads to increased denitrification rates [28,31].

While the majority of the published experiments/applications used common gravel as porous media, Yalcuk *et al*. [51] examined zeolite efficiency in OMW treatment. They reported that the use of zeolite increased organic matter and ammonia removal, which is consistent with the results of other research groups [68,69].

Although VF CWs usually operate under aerobic conditions, the extremely high pollutant loads in agro-industrial wastewater could lead to anoxic/anaerobic conditions. For this reason Poach *et al*. [37] tried to increase DO concentrations in VF CWs by increasing the number of drainage periods. From these experimental results it was concluded that increased drainage periods do not increase pollutant removal efficiencies [37]. To overcome the problem of low DO concentrations, Babatunde and Zhao [65] used VF CWs with tidal flow strategies which can promote oxygen supply. When

Table 5. VF and hybrid CWs treating agro-industrial wastewater treatment by CWs

Ref.	Wastewater type	CW surface area, m^2	Surface load, g/m^2 per day				Removed surface load, g/m^2 per day			
			C	TKN	TP	TSS	C	TKN	TP	TSS
						VF				
[14]	Winery	127–850	23.6–35.2	0.6–2.7	0.08–0.2	–	22.4–33.4	0.4–1.9	–	–
[32]	OMW		15	–	–	–	12.9	–	–	–
[33]	OMW		114.71	–	OP: 2.74	–	82.5	–	OP: 2.6	–
[34]	OMW		6589 Phenols: 997	175	20.0	–	4810 Phenols: 748	131	17.6	–
[41]	Animal farm	0.5	51.7	22.5	1.84	14	31.0	15.8	1.1	13.3
[48]	Animal farm	n.a.	36–474	46–79	8–174	73–1836	21.6–284	20.7–35.6	6.4–139	–
[52]	Cheese whey	0.03	10	–	–	–	3	–	–	–
[65]	Animal farm	575	–	575	–	35		491	–	32.8
						Hybrid				
[15]	Dairy	1990	1.28	0.74	0.10	1.96	1.2	0.68	0.09	1.92
[19]	Animal farm	0.5	51.7	22.5	1.84	14	31.0	15.8	1.1	13.3
[25]	Animal farm	120	–	1.4–1.5	–	–	–	0.98–1.05	–	–
[26]	Vinegar	730	23.4	0.3	0.06	–	67	83	62	–
[28]	Winery	350	30.4	–	–	–	22.3	–	–	–
[31]	Dairy, swine and potato starch	168–2151	24–92	–	–	–	19.2–73.6	–	–	–
[63]	Dairy	80	50–1500	50–1500	1.5–40	20–400	45–1350	32.5–975	0.8–21	18.8–376
[67]	Dairy	1.87	173.5	–	2	22.3	156	–	1.6	20.1

treating wastewaters containing high organic loads, where the oxygen supply from the plant root zone is insufficient, the tidal flow VF CWs increases DO concentration in the biofilm and thus enhances organic matter degradation. Thus organic surface load can increase up to 112g BOD/m^2 per day, and still maintain aerobic conditions [37]. VF CW tolerance to such high organic loads has also been attributed to tidal flow operation and not vegetation as *Phragmites* can only release 0.02 g O$_2$/m^2 per day [37].

The most efficient CW system for agro-industrial wastewater treatment appears to be a hybrid system (Table 5) of both VF and HSF stages. This system has achieved the highest removal rates among all CWs types, ranging from 83 to 96% for organic matter, 55 to 92% for TKN, 52 to 96% for TP, and 83 to 99% for TSS, while receiving high pollutant surface loads (1.28 to 1500 g/m^2 per day for organic matter, 0.3 to 1500 g/m^2 per day for TKN, 0.06 to 40 g/m^2 per day for phosphorus and 1.96 to 400 g/m^2 per day for TSS).

DISCUSSION – DESIGN AND OPERATIONAL SUGGESTIONS

As agro-industrial wastewaters have common characteristics (*i.e.*, high organic loads, low pH values, toxic effects) the CWs treating them present the same problems. Thus, general suggestions can be made on the design and operation of CWs treating agro-industrial wastewaters. The following suggestions concern issues such as temperature, prior-treatment stage, chosen vegetation, porous media and CW plain view.

Temperature usually affects CW performance as the main pollutant mechanism, biological degradation, is temperature-dependent. Lee *et al*. [67] examined organic matter and TSS temporal variations, and found that temperature did not significantly affect their removal. This was also observed by Akratos and Tsihrintzis [70], who state that organic matter degradation is not affected by temperature, because aerobic and anaerobic bacteria responsible for organic matter degradation can function even at low temperatures (5 °C). Contrary to these observations, Newman *et al*. [11] and Mustafa *et al*. [59] reported that nitrogen and phosphorus removal are strongly temperature-dependent. Akratos and Tsihrintzis [70], who also reported a strong correlation between nitrogen removal and temperature, claim that this phenomenon could be attributed to plant uptake and that nitrifying bacteria increase their performance in temperatures above 15 °C.

Concerning vegetation in CW systems treating agro-industrial wastewater, although numerous plant species have been tested, no specific species has been proved to be superior over the others for pollutant removal. The main concern is to select a species resistant to the toxic wastewaters involved and which is indigenous to the geographical area. Tests indicate that the most tolerant plant species is *Phragmites* spp. (the common reed), as it appears resistant to the toxic effects of dairy, animal farm, winery and olive mill wastewaters, and demonstrates high pollution removal efficiencies. Furthermore, it can be found free-growing in most areas. To overcome vegetation species limitations and achieve higher removal rates, Harrington and McInnes [71] propose the use of a variety of vascular plants as long as pollutant concentrations have no toxic effects on vegetation.

Another issue concerning vegetation is the density of the plants sown. Research results show that increased plant density increases pollutant removal. Furthermore, initial dense vegetation could also minimize the adjustment period and possible toxic effects. Therefore, in CWs treating agro-industrial wastewater, the initial planting density should increase from 4 [70] to 6 or 8 plants/m^2, depending on the toxicity of the wastewater concerned. As the plants themselves are responsible for only a small percentage of nutrient removal (around 3%) [11,36], there is no need for their periodic removal. On the contrary, this action would cause a decrease in pollutant removal efficiency, as the oxygen levels in the CW would decrease. The CW should only be replanted when the initial vegetation turns yellow and dies. Removal of dead plants avoids increasing nutrient concentrations in the CW caused by the deposition of decaying plant biomass.

Despite the numerous experiments/applications of CWs treating agro-industrial wastewater, in the majority of cases effluent pollutant concentrations remain above EU recommended limits (Table 6), thus prohibiting its direct use for irrigation or disposal. Effluent concentrations below EU limits were reported only in cases where influent concentrations were very low [2,3,10,12,13,17,59,61]. Therefore, when designing CWs for agro-industrial wastewater treatment various parameters should be considered. Figure 1 presents a decision tree for the design of CWs treating agro-industrial wastewaters and provides a rough tool for scientists and engineers. The first designing step should be the appropriate selection of the prior-treatment stage, as the majority of the experiments/applications presented included either a prior-treatment stage or used diluted wastewaters to eliminate toxic effects on CW vegetation. Depending on the presence of colour and toxic substances and the low or high organic load in agro-industrial wastewaters, prior-treatment stages could include just settling tanks for COD and TSS removal when initial organic loads are low. While biological pre-treatment stages should be used when organic loads are high and toxic substances are present in the wastewaters. We propose the use of biological treatment systems that can tolerate high pollutant loads

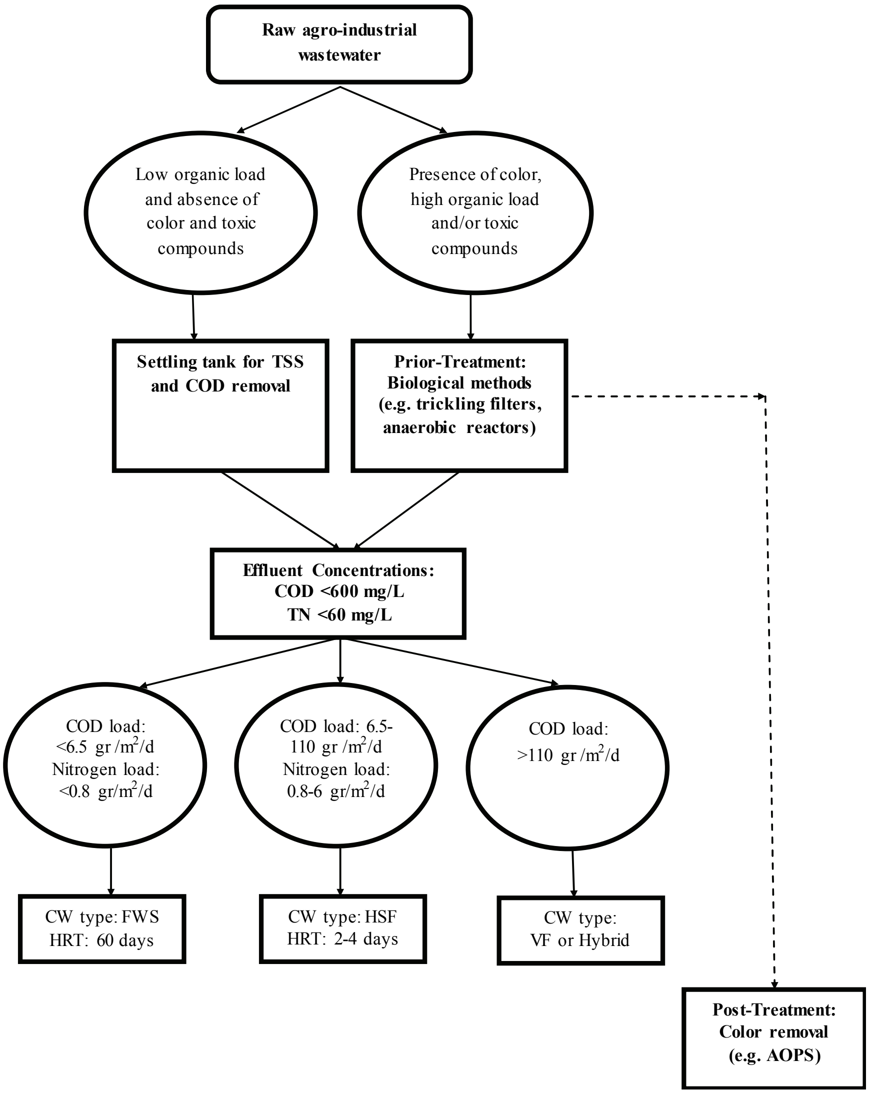


Figure 1. Decision tree for designing CWs treating agro-industrial wastewater.

(*e.g.*, trickling filters, anaerobic reactors and common suspended growth reactors). From the published research it is deduced that CWs were used mainly as polishing treatment stages for agro-industrial wastewater treatment. Bearing in mind that the main advantage of CWs is their low operational cost, they should be coupled with other low-cost treatment technologies (*e.g.*, biological trickling filters, coagulation-flocculation). Biological trickling filters have been successfully used in combination with CWs for OMW treatment [34,72]. The presence of colour in agro-industrial wastewater complicates design as a post-treatment stage should also be included for colour removal. This post-treatment stage usually involves physicochemical methods (*e.g.*, electro-oxidation, ozonation, advanced oxidation processes – AOPS) [33].

Selection of the suitable CW system is also a crucial issue for agro-industrial wastewater treatment. This issue should be considered together with pollutant surface load, as excessive loads negatively affect pollutant removal rates [2,19]. Therefore, CW selection should be based on the maximum pollutant loads that each CW type can tolerate, while effluent concentrations should be below the legislated limits for discharge into a municipal sewerage system. Although all CWs present a wide range of pollutant surface load removal rates (Tables 2–5), design suggestions (Fig. 1) are based only on pollutant loads that lead to effluent concentrations below permitted limits for discharge into a sewer system (Table 6). Specifically, for low pollutant loads (up to 6.5 gr COD/m^2 per day and 0.8 g N/m^2 per day) FWS operating with an HRT of 60 days can achieve effluent concentrations below the permitted limits for discharge into a municipal sewer system (Fig. 1). Cronk [7] has proposed some guidelines for CWs treating wastewater either from animal farms or dairy factories. These guidelines concerned mainly BOD removal and could be characterized as rather conservative, as for a FWS CW

Table 6. Pollutant influent and effluent concentrations compared with EU standards (EU Directive 1991/271/EEC)

Ref.	Influent concentration, mg/L					Effluent concentration, mg/L				
	COD	TKN	$N–NH_4^+$	$N–NO_3^-$	TP	COD	TKN	$N-NH_4^+$	$N–NO_3^-$	TP
EU standards						120		10		2
[2]	150	20	40	4.5	4	15	3.2	0.2	0.14	2.2
[3]	1747	237	188	3.7	37	34	19	14	0.6	17
[10]	682	97	74	0.6	10.4	89	63	25	32	4.7
[11]	2700	102	7.7	0.3	26	611	74	52	0.1	14
[12]	1900	164	72	5.5	53	53	3.3	32	9.9	2.7
[13]	1219	65	22	0.5	13	98	33	25	0.5	5
[18	2240	135	118	0	–	658	34	14	7.5	–
[19]	1034	–	448	–	–	310	–	134	–	–
[23]	–	38	–	–	11	–	23	–	–	7.5
[24]	–	907	366	–	–	–	248	221	–	–
[27]	254	29	18	–	17	158	17	11	–	13
[29]	1160	200	185	3.7	40	190	156	144	1.7	21
[32]	129100	90	–	–	–	95100	560	–	–	–
[34]	14000	506	123	–	95	3500	99	36	–	12
[35]	3220	1333	1072	–	–	2200	333	313	–	–
[37]	445	66	–	–	71	280	30	–	–	65
[39]	796	171	139	0.6	–	471	87	66	0.6	–
[40]	285	296	196	<2	–	277	247	128	<2	–
[41]	2500	–	90	2	40	625	–	60	–	19
[43]	2000	–	0.3	–	5.3	210	–	0.1	2.3	6.4
[44]	1200–3500	–	–	–	–	10–120	–	–	–	–
[45]	3167	104	4.4	101	265	950	26	0.6	9.3	53
[46]	1700	360	–	–	58	115	7.8	–	–	0.8
[51]	2880	–	0.9	–	68	750	–	0.5	–	3.5
[59]	1500	–	40	3.4	–	75	–	0.4	1	–
[62]	–	20–120	–	–	20–50	–	5–15	–	–	10–30
[66]	–	116	86	–	56	–	70	53	–	48

the maximum BOD surface was proposed to be 73 kg/ha per day, with minimum HRT of 12 days.

Subsurface flow CWs (HSF and VF) are more efficient bioreactors and are preferred for the treatment of heavily polluted wastewaters as they can receive high loads of both organic matter and N. Specifically, HSFs appear to be rather effective at agro-industrial wastewater treatment as they can tolerate pollutant loads up to 110 g COD/m^2 per day and 6.5 g N/m^2 per day (Fig. 1). Additionally, the HRTs tested in HSF CWs are significantly lower compared to those used in FWSs and range from 2 to 4 days.

Although VF and hybrid CWs have not been used extensively for agro-industrial wastewater treatment they have been recorded to receive high organic loads (over 110 g COD/m^2 per day), and achieve high removal rates (up to 90%, Table 5). Although final effluents are above permitted limits for discharge into municipal wastewater systems, VF and hybrid CWs should be preferred when organic loads exceed 110 g COD/m^2 per day (Fig. 1; Table 6).

To overcome the limitations of each CW system, several points should be highlighted:

- The main advantage of FWSs is the higher DO concentration observed compared to that of HSF and VF systems. On the other hand, FWS CWs are not preferred for agro-industrial wastewater treatment as they require higher HRTs than HSF and VF CWs. This problem was overcome by Stefanakis and Tsihrintzis [73], who combined FWS and HSF by raising the water level in an HSF system. This new CW type achieved similar removal rates to the HSF system despite receiving 15-20% increased pollutant loads.
- During the operation of many CWs treating agro-industrial wastewaters, plant density was recorded to be higher near the CW inlet. This is attributed to higher nutrient concentrations at and around the inlet. This phenomenon confirms that horizontal flow CWs (FWS and HSF) function as plug-flow reactors and

therefore the biochemical processes occurring are more intense near the inlet. This phenomenon could be exploited to increase CW efficiency in two ways. The first is to change the design of the CW plain view from the common rectangular shape to trapezoidal. This would increase the area near the inlet where nutrient availability is higher, plant density is higher and biochemical processes are more intense. This trapezoidal design was tested by Kotti *et al.* [74], who used FWS CWs for municipal wastewater treatment and found that the trapezoidal CW was cca. 8% more efficient at pollutant removal than the rectangular CW. The second method is to use multiple inlet points along the units. In this way nutrient availability increases along the length of CW, therefore increasing both plant density and pollutant removal. This scenario was tested by Stefanakis *et al.* [75] who used three different inlet points and two different schemes (33:33:33 and 60:25:15). Their results showed that a gradual wastewater inflow from multiple inlet points (60:25:15) significantly increased pollutant removal efficiencies (up to 5%).

Another CW design issue is the origin of the soil (FWS) or porous media (HSF and VF) used. The type of soil/porous media is important as it could positively affect N and P removal. Most studies have not examined thoroughly the effect of CW substrate, however one attempt was made to use zeolite [68]. Zeolite, which is a natural absorbent, has also been used as a substrate in post-treatment filters that increase organic matter and ammonia removal [68,69]. Other substrate materials tested include bauxite, flying ash, river gravel and quarry gravel [68–70,76,77]. From the literature it can be concluded that substrate origin and chemical composition is critical, as minerals with reactive Fe and Al or calcareous materials that promote Ca phosphate precipitation are rather efficient at phosphorus removal, and materials with high cation exchange ability promote ammonium removal.

The major problem in CW application is porous media clogging, especially when treating wastewaters containing high TSS concentrations, such as agro-industrial wastewaters. Future research should focus on testing materials that would increase CW porosity and thus limit the clogging effect. These materials could include the plastic materials already used in biological trickling filters [72], where biofilm density is higher than in CWs.

CONCLUSIONS

CWs appear to be rather effective in treating agro-industrial wastewaters after a prior treatment stage. Although CWs were originally designed to receive low pollutant loads they have been proved to tolerate extremely high pollutant loads and still achieve high pollutant removal rates. Although the presence of vegetation positively influences pollutant removal, different species do not significantly affect CW performance. Based on the above literature review, some initial design guidelines for CWs treating agro-industrial wastewaters can be suggested including:

a) Prior treatment stages are imperative before the CW stage. When initial organic loads are low and no toxic substances are present in the wastewater, a simple settling tank suffices for COD and TSS removal. However, when organic loads are high and toxic substances are present, a biological treatment system should be used before the CW stage.

b) To achieve effluent concentrations that meet permitted limits for discharge into a municipal sewerage system, the appropriate CW system and HRT should be selected according to the organic and N loads of the wastewaters concerned.

c) A post-treatment stage is imperative in cases where colour is present in the wastewaters.

d) To further improve CW performance on agro-industrial wastewater treatment specific design and operation parameters should be examined, including CW plain, vegetation density, step feeding, special porous media, etc.

Acknowledgement

The first author gratefully acknowledges a graduate studies scholarship from the Hellenic State Scholarships Foundation.

REFERENCES

[1] P.G. Hunt, T.A. Matheny, A.A. Szogi, Denitrification in constructed wetlands used for treatment of swine wastewater, J. Environ. Qual. **32** (2003) 727–735.

[2] D. Ghosh, B. Gopal, Effect of hydraulic retention time on the treatment of secondary effluent in a subsurface flow constructed wetland, Ecol. Eng. **36** (2010) 1044–1051.

[3] R. Jamieson, R. Gordon, N. Wheeler, E. Smith, G. Stratton, A. Madani, Determination of first order rate constants for wetland treating livestock wastewater in cold climates, J. Env. Eng. Sci. **6** (2007) 65–72.

[4] A.M. Ibekwe, C.M. Grieve, S.R. Lyon, Characterization of microbial communities and composition in constructed dairy wetland wastewater effluent, Appl. Environ. Microbiol. **69** (2003) 5060–5069.

[5] R. Kadlec, S. Wallace, Treatment Wetlands, 2nd ed., CRC Press, Boca Raton, FL, 2009.

[6] M.H. Awady, R.A. Wahaab, Agro-industry wastewater treatment, Environ. Technol. **20** (1999) 1001–1004.

[7] J.K. Cronk, Constructed wetlands to treat wastewater from dairy and swine operations: a review, Agric. Ecosyst. Environ. **58** (1996) 97–114.

[8] J. Vymazal, Constructed wetlands for treatment of industrial wastewaters: A review, Ecol. Eng. **73** (2014) 724––751.

[9] J.M. Newman, J.C. Clausen, Seasonal effectiveness of a constructed wetland for processing milkhouse wastewater, Wetlands **17** (1997) 375–382.

[10] J. Kern, C. Idler, G. Carlow, Removal of fecal coliforms and organic matter from dairy farm wastewater in a constructed wetland under changing climate conditions, J. Environ. Sci. Heal., A **35** (2000) 1445–1461.

[11] J.M. Newman, J.C. Clausen, J.A. Neafsey, Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut, Ecol. Eng. **14** (2000) 181–198.

[12] J.A. Schaafsma, A.H. Baldwin, C.A. Streb, An evaluation of a constructed wetland to treat wastewater from a dairy farm in Maryland, USA, Ecol. Eng. **14** (2000) 199––206.

[13] P. Mantovi, M. Marmiroli, E. Maestri, S. Tagliavini, S. Piccinini, N. Marmiroli, Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlor wastewater, Bioresour. Technol. **88** (2003) 85–94.

[14] F. Masi, G. Conte, N. Martinuzzi, B. Pucci, Winery high organic content wastewater treated by constructed wetlands in Mediterranean climate, in Proceedings of IWA 8th International Conference on wetlands systems for water pollution control, Arusha, Tanzania, 2002, pp. 1–9.

[15] W. Browne, P.D. Jenssen, Exceeding tertiary standards with a pond/reed bed system in Norway, Water Sci. Technol. **51** (2005) 299–306.

[16] E.J. Dunne, N. Culleton, G. O'Donovan, R. Harrington, A.E. Olsen, An integrated constructed wetland to treat contaminants and nutrients from dairy farmyard dirty water. Ecol. Eng. **24** (2005) 221–234.

[17] V. Gasiunas, Z. Strusevicius, M.S. Struseviciene, Pollutant removal by horizontal subsurface flow constructed wetlands in Lithuania, J. Environ. Sci. Heal., A **40** (2005) 1467–1478.

[18] R.H. Kadlec, C.C. Tanner, V.M., Hally, M.M. Gibbs, Nitrogen spiraling in subsurface-flow constructed wetlands: Implications for treatment response, Ecol. Eng. **25** (2005) 365–381.

[19] S. Kantawanichkul, S. Somprasert, Using a compact combined constructed wetland system to treat agricultural wastewater with high nitrogen, Wat. Sci .Technol. **51** (2005) 47–53.

[20] E. Smith, R. Gordon, A. Madani, G. Stratton, Cold climate hydrological flow characteristics of constructed wetlands, Can. Biosyst. Eng. **47** (2005) 1–7.

[21] P. Munoz, A. Drizo, W.C. Hession, Flow patterns of dairy wastewater constructed wetlands in a cold climate, Water Res. **40** (2006) 3209–3218.

[22] P.D. Sindilariu, A. Brinker, R. Reiter, Factors influencing the efficiency of constructed wetlands used for the treatment of intensive trout farm effluent, Ecol. Eng. **35** (2009) 711–722.

[23] C.C. Tanner, J.S. Clayton, M.P. Upsdell, Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands – *i.* removal of oxygen demand, suspended solids and faecal coliforms, Water Res. **29** (1995) 17–26.

[24] R.L. Knight, V.W.E.J. Payne, R.E. Borer, R.A.J. Clarke, J.H. Pries, Constructed wetlands for livestock wastewater management, Ecol. Eng. **15** (2000) 41–55.

[25] M.E. Poach, P.G. Hunt, M.B. Vanotti, K.C. Stone, T.A. Matheny, M.H. Johnson, E.J. Sadler, Improved nitrogen treatment by constructed wetlands receiving partially nitrified liquid swine manure, Ecol. Eng. **20** (2000) 183––197.

[26] M.Z. Justin, D. Vrhovsek, A. Stuhlbacher, T.G. Bulc, Treatment of wastewater in hybrid constructed wetland from the production of vinegar and packaging of detergents, Desalination **246** (2009) 100–109.

[27] V.R. Moreira, B.D. LeBlanc, E.C. Achberger, D.G. Frederick, C. Leonardi, Design and evaluation of a sequential biological treatment system for dairy parlor wastewater in southeastern Louisiana, Appl. Eng. Agric. **26** (2010) 125–136.

[28] L. Serrano, D. de la Varga, I. Ruiz, M. Soto, Winery wastewater treatment in a hybrid constructed wetland, Ecol. Eng. **37** (2011) 744–753.

[29] C.Y. Lee, C.C. Lee, F.Y. Lee, S.S. Tseng, C.J. Liao, Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads, Bioresour. Technol. **92** (2004) 173–179.

[30] Y.Q. Zhao, A.O. Babatunde, Y.S. Hu, J.L.G. Kumar, X.H. Zhao, Pilot field-scale demonstration of a novel alum sludge-based constructed wetland system for enhanced wastewater treatment, Process Biochem. **46** (2011) 278–283.

[31] K. Kato, T. Inoue, H. Ietsugu, T. Kobad, H. Sasaki, N. Miyaji, K. Kitagaw, P.K. Sharm, T. Nagasaw, Performance of six multi-stage hybrid wetland systems for treating high-content wastewater in the cold climate of Hokkaido, Japan, Ecol. Eng. **51** (2013) 256–263

[32] M.D. Bubba, L. Checchini, C. Pifferi, L. Zanieri, L. Lepri, Olive mill wastewater treatment by a pilot-scale subsurface horizontal flow (SSF-h) constructed wetland, Anal. Chim. **94** (2004) 875–887.

[33] P. Grafias N.P. Xekoukoulotakis, D. Mantzavinos, E. Diamadopoulos, Pilot treatment of olive pomace leachate by vertical-flow constructed wetland and electrochemical oxidation: An efficient hybrid process, Water Res. **44** (2010) 2773–2780.

[34] E. Herouvim, C.S. Akratos, A.G. Tekerlekopoulou, D.V. Vayenas, Treatment of olive mill wastewater in pilot-scale vertical flow constructed wetlands, Ecol. Eng. **37** (2011) 931–939.

[35] M. Trias, Z. Hu, M.M. Mortula, R.J. Gordon, G.A. Gagnon, Impact of seasonal variation on treatment of swine wastewater, Environ. Technol. **25** (2004) 775––781.

[36] R. Gottschall, C. Boutin, A. Crolla, C. Kinsley, P, Champagne, The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater Ontario, Canada, Ecol. Eng. **29** (2007) 154–163.

[37] M.E. Poach, P.G. Hunt, G.B. Reddy, K.C. Stone, M.H. Johnson, A. Grubbs, Effect of intermittent drainage on swine wastewater treatment by marsh-pond-marsh constructed wetlands, Ecol. Eng. **30** (2007) 43–50.

[38] T.Y. Yeh, C.C. Chou, C.T. Pan, Heavy metal removal within pilot-scale constructed wetlands receiving river water contaminated by confined swine operations, Desalination **249** (2009) 368–373.

[39] P.G. Hunt, M.E. Poach, T.A. Matheny, G.B. Reddy, K.C. Stone, Denitrification in marsh-pond-marsh constructed wetlands treating swine wastewater at different loading rates, Soil Sci. Soc. Am. J. **70** (2006) 487–493.

[40] E. Shamir, T.L. Thompson, M.M. Karpiscak, R.J. Freitas, J. Zauderer, Nitrogen accumulation in a constructed wetland for dairy wastewater treatment, J. Am. Water Resour. AS. **37** (2001) 317–325.

[41] Y.Q. Zhao, G. Sun, S.J. Allen, Anti-sized reed bed system for animal wastewater treatment: a comparative study, Water Res. **38** (2004) 2907–2917.

[42] I.E. Kapellakis, V. Paranychianakis, K.P. Tsagkarakis, A.N. Angelakis, Treatment of olive mill wastewater with constructed wetlands. Water (Switzerland) **4** (2012) 260–271.

[43] S.E. Moir, I. Svoboda, G. Sym, J. Clark, M.B. McGechan, K. Castle, An experimental plant for testing methods of treating dilute farm effluents and dirty water, Biosyst. Eng. **90** (2005) 349–355.

[44] M.Y. Sultana, C. Mourti, T. Tatoulis, C.S. Akratos, A.G. Tekerlekopoulou, D.V. Vayenas, Effect of hydraulic retention time, temperature, and organic load on a horizontal subsurface flow constructed wetland treating cheese whey wastewater, J. Chem. Technol. Biotechnol. 2015, DOI: 10.1002/jctb.4637.

[45] E. Meers, D.P.L. Rousseau, N. Blomme, E. Lesage, G. Du Laing, F.M.G. Tack, M.G. Verloo, Tertiary treatment of the liquid fraction of pig manure with Phragmites australis, Water Air Soil Poll. **160** (2005) 15–26.

[46] E. Meers, F.M.G. Tack, I. Tolpe, E. Michels, Application of a full-scale constructed wetland for tertiary treatment of piggery manure: Monitoring results, Water Air Soil Poll. **193** (2008) 15–24.

[47] D. de la Varga, I. Ruiz, M. Soto, Winery wastewater treatment in subsurface constructed wetlands with different bed depths, Water Air Soil Poll. **224** (2013) 1485–1498.

[48] E.S. Aktas, S. Imre, L. Ersoy, 2001. Characterization and lime treatment of olive mill wastewater, Water Res. **35** (2013) 2336–2340.

[49] E.J. Olguın, G.E. Sanchez-Galvan, R.E. Gonzalez-Portela, M. Lopez-Vela, Constructed wetland mesocosms for the treatment of diluted sugarcane molasses stillage from ethanol production using *Pontederia sagittata*, Water Res. **42** (2008) 3659–3666.

[50] A. Dordio, A.J.P. Carvalho, Constructed wetlands with light expanded clay aggregates for agricultural wastewater treatment, Sci. Total Environ. **463–464** (2013) 454–461.

[51] A. Yalcuk, N.B. Pakdil, S.Y. Turan, Performance evaluation on the treatment of olive mill waste water in vertical subsurface flow, constructed wetlands, Desalination **262** (2010) 209–214.

[52] A. Yalcuk, The macro nutrient removal efficiencies of a vertical flow constructed wetland fed with demineralized cheese whey powder solution, Int. J. Phytoremed. **14** (2012) 114–127.

[53] F.A. Comin, J.A. Romero, V. Astorga, G. Garcia, Nitrogen removal and cycling in restored wetlands used as filters of nutrients for agricultural runoff, Water Sci. Technol. **35** (1997) 255–261.

[54] M. Greenway, A. Woolley, Changes in plant biomass and nutrient removal over 3 years in a constructed free water surface flow wetland in Cairns Australia, in Proceedings of 7th International Conference on Wetland Systems for Water Pollution Control, Lake Buena Vista, Florida, 2000, pp. 707–718.

[55] J.I. Hansen, F.O. Anderson, Effects of *Phragmites australis* roots and rhizomes on redox potentials, nitrification and bacterial numbers in the sediment, in Proceedings of the 9th Nordic Symposium on sediments, Malme, Sweden, 1981, pp. 72–88.

[56] K.R. Reddy, W.H. Patrick, C.W. Lindau, Nitrification-denitrification at the plant root-sediment interface in wetlands, Limnol. Oceanogr. **34** (1989) 1004–1013.

[57] J.M. Tiedje, Ecology of denitrification and dissimilatory nitrate reduction to ammonium, in: A.J.B. Zehnder (Ed.), Biology of Anaerobic Microorganisms. John Wiley & Sons, New York, 1988, pp. 179–244.

[58] S. Dipu, A.A. Kumar, V.S.G. Thanga, Phytoremediation of dairy effluent by constructed wetland technology, Environmentalist **31** (2011) 263–278.

[59] A. Mustafa, M. Scholz, R. Harrington, P. Carroll, Long-term performance of a representative integrated constructed wetland treating farmyard runoff, Ecol. Eng. **35** (2009) 779–790.

[60] C.C. Tanner, M.L. Nguyen, J.P.S. Sukias, Nutrient removal by a constructed wetland treating subsurface drainage from grazed dairy pasture, Agric. Ecosyst. Environ. **105** (2005) 145–162.

[61] P.G. Hunt, K.C. Stone, T.A. Matheny, M.E. Poach, M.B. Vanotti, T.F. Ducey, Denitrification of nitrified and non-nitrified swine lagoon wastewater in the suspended sludge layer of treatment wetlands, Ecol. Eng. **35** (2009) 1514–1522.

[62] J.M. Hathaway, M.J. Cook, R.O. Evans, Nutrient removal capability of a constructed wetland receiving groundwater contaminated by swine lagoon seepage, Am. Soc. Agr. Biol. Eng. **53** (2010) 1–9.

[63] D.P.L. Rousseau, P.A. Vanrolleghem, N. De Pauw, Constructed wetlands in Flanders: a performance analysis, Ecol. Eng. **23** (2004) 151–163.

[64] A.M. Farnet, P. Prudent, F. Ziarelli, M. Domeizel, R. Gros, Solid-state 13C NMR to assess organic matter transformation in a subsurface wetland under cheese-dairy farm effluents, Bioresour. Technnol. **100** (2009) 4899–4902.

[65] A.O. Babatunde, Y.Q. Zhao, Two strategies for improving animal farm wastewater treatment in reed beds, Environ. Technol. **31** (2010) 1343–1348.

[66] K.C. Stone, M.E. Poach, P.G. Hunt, G.B. Reddy, Marsh-pond-marsh constructed wetland design analysis for swine lagoon wastewater treatment, Ecol. Eng. **23** (2004) 127–133.

[67] M.S. Lee, A. Drizo, D.M. Rizzo, G. Druschel, N. Hayden, E. Twohig, Evaluating the efficiency and temporal variation of pilot-scale constructed wetlands and steel slag phosphorus removing filters for treating dairy wastewater, Water Res. **44** (2010) 4077–4086.

[68] A.I. Stefanakis, V.A. Tsihrintzis, Effects of loading, resting period, temperature, porous media, vegetation and aeration on performance of pilot-scale vertical flow constructed wetlands, Chem. Eng. J. **181**–**182** (2012) 416––430.

[69] J. Vymazal, Plants used in constructed wetlands with horizontal subsurface flow: a review, Hydrobiologia **674** (2011)133–156.

[70] A.I. Stefanakis, V.A. Tsihrintzis, Use of zeolite and bauxite as filter media treating the effluent of Vertical Flow Constructed Wetlands, Micropor. Mesopor. Mat. **155** (2012) 106–116.

[71] C.S. Akratos, V.A. Tsihrintzis, Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands, Ecol. Eng. **29** (2007) 173–191.

[72] R. Harrington, R. McInnes, Integrated constructed wetlands (ICW) for livestock wastewater management, Bioresour. Technol. **100** (2009) 5498–5505.

[73] M. Michailides, P. Panagopoulos, C.S. Akratos, A.G. Tekerlekopoulou, D.V. Vayenas, A full-scale system for aerobic biological treatment of olive mill wastewater, J. Chem. Technol. Biotechnol. **86** (2011) 888–892.

[74] A.I. Stefanakis, V.A. Tsihrintzis, Effect of outlet water level raising and effluent recirculation on removal efficiency of pilot-scale, horizontal subsurface flow constructed wetlands, Desalination **248** (2009) 961–976.

[75] I.P. Kotti, G.D. Gikas, V.A. Tsihrintzis, Effect of operational and design parameters on removal efficiency of pilot-scale FWS constructed wetlands and comparison with HSF systems, Ecol. Eng. **36** (2010) 862–875.

[76] A.I. Stefanakis, C.S. Akratos, V.A. Tsihrintzis, Effect of wastewater step-feeding on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands, Ecol. Eng. **37** (2011) 431–443.

[77] A. Drizo, C.A. Frost, J. Grace, K.A. Smith, Physico-chemical screening of phosphate-removing substrates for use in constructed wetland systems, Water Res. **33** (1999) 3595–3602.

[78] C.A. Arias, M.D. Bubba, H. Brix, Phosphorus removal by sands for use as media in subsurface flow constructed reed beds, Water Res. **35** (2001) 1159–1168.

IZVOD

UPOTREBA PLANSKIH MOČVARA U TRETMANU AGROINSTUSTRIJSKIH OTPADNIH VODA: PREGLED

Mar-Yam Sultana[1], Christos S. Akratos[1], Dimitrios V. Vayenas[1,2], Stavros Pavlou[2,3]

[1]*Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece*
[2]*Institute of Chemical Engineering Sciences, FORTH, Patras, Greece*
[3]*Department of Chemical Engineering, University of Patras, Patras, Greece*

(Pregledni rad)

Širom sveta se planske močvare upotrebljavaju u tretmanu otpadnih voda zbog svoje jednostavnosti i niskih troškova izrade. Njihova upotreba se više ne ograničava samo na komunalne otpadne vode, već se širi i na tretman jako zagadjenih otpadnih voda kao što su agro industrijske otpadne vode. Ovaj rad pruža sveoubhvatan pregled literature koja se bavi aplikacijom planskih močvara za potrebe tretiranja različitih agro industrijskih otpadnih voda, kao i pitanja nagomilanog otpada na površini vode, ulogu tipova konstruisanih tresetišta, faze pre samog tretmana, biljne vrste koje su efikasne u otklanjanju zagađivača. Rezultati ukazuju da konstruisana tresetišta mogu da tolerišu visok nivo zagadjivača i toksičnih supstanci bez gubljenja svojih osobina, i na taj način su ovi sistemi veoma efikasni kao bio reaktori u zagadjenom okruženju. Ovaj rad predlaže pitanja koja mogu da poboljšaju efikasnost tretmana i predlaže dizajn i predloge u vidu odgovarajuće vegetacije, poroznih materijala i pregleda konstruisanih tresetišta. Na kraju se predlaže šematski prikaz kao pomoć u dizajniranju tresetišta neophodnih za tretiranje agro industrijskih otpadnih voda koji treba da posluži kao alat za naučnike i inžinjere.

Ključne reči: Konstruisana tresetišta • Agroindustrijske otpadne vode • Pregled • Šematski prikaz • Dizajn

Nivoi makroelemenata i toksičnih elemenata u biljnim čajevima

Željko A.Mihaljev[1], **Željko. N. Ćupić**[2], **Milica M. Živkov-Baloš**[1], **Sandra M. Jakšić**[1]

[1]Naučni institut za veterinarstvo-„Novi Sad“, Novi Sad, Srbija
[2]Naučni institut za reprodukciju i v.o. domaćih životinja,Temerin, Srbija

Izvod

Ukupno je ispitano 14 različitih vrsta čajeva, i to: hajdučka trava, bosiljak, kantarion, pitoma nana, rastavić, kopriva, majkina dušica, kukuruzna svila, hibiskus, beli slez, kamilica, šipak-divlja ruža, vrijesak i divlja nana. Uzorci za merenje pripremljeni su metodom mikrotalasne digestije, a merenje je izvršeno sledećim metodama: masenom spektrometrijom sa indukovano spregnutom plazmom (Cd, Hg, Pb, As i Sb); atomskom apsorpcionom spektrometrijom (Mg); emisionom plamenom fotometrijom (Ca, K i Na) i spektrofotometrijom (P). Intervali varijacija (mg/kg) za makroelemente bili su: Ca (2738–35399); P (1545–6264); Mg (1647–7816); Na (293–10629) i K (9683–33985), a za toksične elemente: Cd (0,014–0,645); Hg (<0,001–0,017); Pb (0,064–1,340); As (0,030–0,544) i Sb (0,004–0,068). Treba istaći tri uzorka: hajdučka trava i dva uzorka kantariona u kojima je izmerena vrednost Cd (0,499±±0,001; 0,539±0,002 i 0,645±0,003 mg/kg, redom) veća od 0,3 mg/kg, a koja predstavlja preporučeni dozvoljeni gornji nivo Cd u osušenom biljnom materijalu. Primenjena je Ward metoda hijerarhijske klaster analize, u cilju grupisanja biljnih čajeva po količini toksičnih elemenata.

Ključne reči: biljni čajevi, makroelementi, toksični elementi, ICP-MS, AAS.

Dostupno na Internetu sa adrese časopisa: http://www.ache.org.rs/HI/

NAUČNI RAD

UDK 633.88:663.95.519.237.8:543

Hem. Ind. **69** (2) 143–153 (2015)

doi: 10.2298/HEMIND130424029M

Mineralne materije u životu biljaka imaju višestruku ulogu. Sadržaj minerala u suvoj materiji biljaka u proseku se kreće od 1–6%. U biljkama se nalaze u vidu jona, neorganskih i organskih soli i ugrađeni u razna organska jedinjenja [1]. Učestvuju u katalizi brojnih hemijskih reakcija biljaka. S obzirom na zastupljenost u izgradnji biljaka, grupišu se na: makro, mikro i ultramikroelemente. U makroelemente se ubrajaju: C, O, H, N, P, K, Ca, Mg, S, Na, Si i Cl i njihov sadržaj , ako se izuzmu C, O i H, u suvoj materiji biljaka u proseku se kreće od 2–60 mg/g. Sadržaj mikroelemenata (Cu, Zn, B, Mn i dr.) u suvoj materiji biljaka je manji od 1 mg/g i obično je veći od 1μg/g, dok je sadržaj ultramikroelemenata (J, Se, Mo i dr.) manji od 1μg/g [2]. Treba istaći, da ovakva podela ne odražava njihovu ulogu u metabolizmu biljaka. Tako, jedna grupa elemenata (teški metali), među kojima ima i esencijalnih mikroelemenata (Fe, Zn, Cu, Mn, Co i dr.) pri većim koncentracijama deluju toksično na biljke, a što se manifestuje u poremećaju jednog ili većeg broja metaboličkih i/ili hemijskih reakcija [3]. Količina mineralnih materija u biljkama znatno varira i ovo variranje često je veće nego kod pojedinih grupa organskih materija [4]. Varijacije u sadržaju mineralnih materija u biljkama, posledica su različitih faktora od kojih su najvažniji: biljna vrsta, starost zasada, pedološke karakteristike zemljišta i primena agrotehničkih mera [5–7].

Kada su u pitanju agrotehničke mere, prvenstveno se misli na upotrebu pesticida i veštačkog đubriva. Tako, korišćenje bakarnih fungicida, uzrokuje prisustvo neželjenih koncentracija bakra i povećava akumulaciju olova i kadmijuma u crnom čaju [8].

Biljke su direktan ili indirektan izvor minerala u ljudskoj ishrani. Poseban značaj za čoveka imaju biljne vrste koje služe za dobijanje fitopreparata u farmaceutskoj industriji u formi monokomponentnih čajeva ili čajnih mešavina, koje imaju veliku primenu u tradicionalnoj medicini. Kao izvor za dobijanje lekovite sirovine služe samonikle biljke iz prirode (preko 200), odnosno plantažno gajene biljke kojih ima oko 30 [9]. Lekovite biljke se uglavnom koriste u formi čajeva. Čaj je najpopularniji napitak u svetu, čije se konzumiranje zbog nekoliko esencijalnih sastojaka smatra blagotvornim. Bez obzira da li se radi o crnom, zelenom ili nekom „biljnom čaju“, ovo piće je veoma prisutno u svakidašnjem životu ljudi, a na to ukazuje i njegova velika proizvodnja i potrošnja. Hemijski sastav čajeva je veoma složen i u njima nalazimo flavonoide, alkaloide, enzime, minerale,elemente u tragovima i drugo [10]. U Srbiji, čajevi se uglavnom pripremaju od aromatičnih biljnih vrsta, koje sadrže etarska ulja, koja osim što su lekovita, imaju prijatan ukus i miris. Ljudski organizam ima potrebu za mineralima unutar odgovarajućih koncentracija, radi normalnog održavanja životnih funkcija. U skladu s tim deficit ili višak esencijalnih teških metala (Fe, Cu, Zn, Co, Mn i Mo), u ishrani može izazvati štetne efekte u organizmu [11,12]. Takođe, toksični elementi (Hg, As, Pb i Cd) koji su prisutni u nekim biljkama, mogu teško oštetiti hemopoetski, imuni, nervni i reproduk-

---

Prepiska: Ž. Mihaljev, Naučni Institut za veterinarstvo „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Srbija.
E-pošta: zeljko@niv.ns.ac.rs

Rad primljen: 24. april, 2013
Rad prihvaćen: 9. april, 2014

tivni sistem. Ovi elementi imaju svojstvo da se akumuliraju, jer ne mogu u potpunosti da se ekskretuju iz organizma [10]. Upravo zbog toga, sadržaj teških metala je jedan od kriterijuma za korišćenje biljnog materijala u proizvodnji tradicionalnih lekova i biljnih infuzija [13].

Primena intenzivnih agrotehničkih mera u savremenoj poljoprivrednoj proizvodnji, blizina industrijskih postrojenja, rudnika, saobraćajnica, neminovno dovodi do kontaminacije zemljišta i biljaka sa pesticidima i teškim metalima. Zbog ovoga nameće se potreba stalne i organizovane kontrole zdravstvene ispravnosti biljaka koje se koriste kao sirovina u farmaceutskoj industriji.

Ispitivanje uzoraka različitih biljnih čajeva, uzetih iz maloprodajnih objekata sa područja grada Novog Sada, kao i uzoraka sa prirodnih staništa (region istočne Srbije, lokalitet I i II), imala su za cilj određivanje sadržaja različitih elemenata, a posebno toksičnih elemenata, kako bi se dobio uvid u bezbednost ovih fitoproizvoda, a imajući u vidu njihovu široku primenu u narodnoj terapiji i potencijalnu mogućnost da akumuliraju toksične elemente.

EKSPERIMENTALNI DEO

Materijal

Uzorci čajeva su prikupljeni iz maloprodajnih objekata sa područja Novog Sada,u njihovom originalnom pakovanju (uzorci 1 do 13), a 3 uzorka lekovitog bilja (uzorci 14 do 16) su uzorkovani sa prirodnih staništa (region istočne Srbije; lokalitet I i II). Iz tabele 1 je vidljivo da se radi o biljnim čajevima čija je primena među stanovništvom Srbije veoma raširena i popularna u narodnoj medicini.

Metode rada

Uzorci za merenje pripremljeni su metodom mikrotalasne digestije u sistemu Ethos, Microwave Labstation, Milestone [15]. Razaranje je izvršeno sa 8 ml razblažene HNO_3 (2:1) i 2ml H_2O_2 (30%) na $t_{\max} = 180\ ^{\circ}C$. Zadati program mikrotalasne digestije ukupno je trajao 30 min. sa maksimalnom snagom od 1000 W. Arsen (He-M, IT 1 s/P), kadmijum (NoG-M, IT 1 s/P), antimon (NoG-M, IT 0.1 s/P), živa (NoG-M, IT 1 s/P) i olovo (NoG-M, IT 0.1 s/P) određeni su tehnikom indukovano-spregnute plazme sa masenom spektrometrijom na instrumentu Agilent ICP-MS 7700 preko izotopa: ^{75}As, ^{111}Cd, ^{121}Sb, ^{201}Hg i ^{208}Pb. Magnezijum ($\lambda = 202{,}6$ nm), je određen atomskom apsorpcionom spektrofotometrijom na instrumentu Varian SpectrAA-10 uz korišćenje D_2-lampe kao background korektora. Natrijum ($\lambda = 330{,}3$ nm), kalijum ($\lambda = 766{,}5$ nm) i kalcijum ($\lambda = 422{,}7$ nm) određeni su metodom emisione plamene fotometrije (Jenway PFP7), a fosfor ($\lambda = 430{,}0$ nm) je određen spektrofotometrijski (Anthelie Advanced, Secomam). Za sve kalibracione krive korišćeni su certifikovani standardi AccuTrace^{TM} Reference Standard (USA) koncentracije 1000 μg/ml. Za utvrđivanje efikasnosti mikrotalasne digestije (tabela 2) korišćeni su sertifikovani referentni materijali: STRAW (IPE 881) i Wheat Flour (NCS ZS 80002b).

Merenje svakog uzorka vršeno je tri puta. Izmerene vrednosti sadržaja elemenata u uzorcima biljnih čajeva, izražene su u mg/kg suve materije pošto je prethodno u svakom biljnom čaju određen sadržaj vlage sušenjem određene količine uzorka na 105 °C.

Tabela 1. Nazivi lekovitih biljaka i delovi biljaka koji su korišćeni u ispitivanju [9,14]
Table 1. The names of medicinal plants and plant parts used in research [9,14]

Red. broj	Biljka	Latinski naziv biljke (Familija)	Lekoviti deo
1	Hajdučka trava	*Achillea millefolium L. (Asteraceae)*	Cela biljka
2	Bosiljak	*Ocimum basilicum L (Lamiaceae)*	Cela biljka
3	Kantarion	*Hypericum perforatum L. (Hypericaceae)*	Cela biljka
4	Pitoma nana	*Mentha x piperita L. (Lamiaceae)*	List
5	Rastavić	*Equisetum arvense L. (Equisetaceae)*	Cela biljka
6	Kopriva	*Urtica dioica L. (Urticaceae)*	Koren
7	Kopriva	*Urtica dioica L. (Urticaceae)*	List
8	Majkina dušica	*Thymus serpyllum L. (Lamiaceae)*	Cela biljka
9	Kukuruz	*Zea mays, Maydis stigma (Poaceae)*	Svila
10	Hibiskus	*Hibiscus sabdariffa L. (Malvaceae)*	Cvet
11	Beli slez	*Althaea officinalis L. (Malvaceae)*	Koren
12	Kamilica	*Matricaria chamomilla L. (Asteraceae)*	Cvet
13	Šipak,divlja ruža	*Rosa canina L. (Rosaceae)*	Plod
14	Vrijesak	*Satureja montana L. (Lamiaceae)*	Cela biljka
15	Kantarion	*Hypericum perforatum L. (Hypericaceae).*	Cela biljka
16	Divlja nana	*Mentha spicata L. (Lamiaceae)*	Cela biljka

Tabela 2. Efikasnost mikrotalasne digestije i limiti detekcije dobijeni merenjem dva sertifikovana referentna materijala
Table 2. Certified and measured values for two certified reference food materials. recoveries are dependent on digestion efficiency as well as analytical accuracy

Ispitivani element	Sertifikovana vrednost, μg/g	Izmerena vrednost, μg/g	Prinos, %	Granica detekcije, μg/g
^{111}Cd[a]	0.150±0.040	0.141±0.039	94	0.001
^{201}Hg[b]	0.082±0.072	0.075±0.059	92	0.001
^{208}Pb[a]	0.340±0.130	0.356±0.116	105	0.001
^{75}As[a]	0.320± 0.070	0.333±0.077	104	0.001
Mg[a]	616±44	579±39	94	1
Ca[a]	402±31	370±25	92	25
K[a]	2370±101	2451±110	103	1
Na[a]	8.30±1.10	7.57±0.91	91	1
P[a]	1770±100	1699±88	96	100

[a]Certified Reference Material: 202-ZC 80002b-Wheat Flour, China National Analysis Center; [b]certified Reference Material: IPE 881, Wheat Straw, Wageningen University, Environmental Sciences

Statistička obrada podataka

Obuhvata određivanje osnovnih parametara statističke analize: aritmetička sredina i standardna devijacija (*SD*). Grupisanje biljnih čajeva obzirom na sadržaj toksičnih elemenata izvršeno je na osnovu Wardove metode hijerarhijske klaster analize, primenjene na standardizovane podatke, na osnovu Euklidskog odstojanja.Vizuelizacija klasterovanja data je pomoću dendrograma [16]. Takođe određeni su koeficijenti korelacije unutar sadržaja toksičnih elemenata u uzorcima biljnih čajeva. Pri ovome korišćen je softverski program Statistika-10, Stat Soft, Inc.

REZULTATI I DISKUSIJA

U tabeli 3 dati su rezultati merenja sadržaja makroelemenata u ispitivanim čajevima.

Makroelementi imaju veliki značaj za biljke, životinje i čoveka. Kalcijum, fosfor, magnezijum i kalijum spadaju u neophodne makroelemente za više biljke, dok se natrijum ubraja u korisne elemente [1,2]. Prosečan sadržaj Ca, Mg, P, K i Na u suvoj materiji biljaka iznosi: 0,5; 0,2; 0,2; 1,0 i 0,01 do 2%, redom [2,17]. Rezultati našeg istraživanja ukazuju na prisustvo visokog sadržaja makroelemenata u biljnim čajevima, što je bilo i očekivano. Ovo ukazuje na povećane potrebe biljaka za ovim elementima i samim tim na njihov značaj za biljni organizam. Takođe, ovo implicira i njihovu veliku mobilnost, bioraspoloživost u zemljištu i dobru

Tabela 3. Sadržaj makroelemenata u uzorcima biljnih čajeva (aritmetička sredina±SD), mg/kg suve materije
Table 3. Content of macroelements in the herbal teas samples (mean±SD), mg/kg dry matter

Red. broj	Biljka	Element				
		Ca	P	Mg	Na	K
1.	Hajdučka trava, cela biljka	6191±99	3220±71	2573±23	293±2,64	23218±302
2.	Bosiljak, cela biljka	17780±284	4107±90	7816±171	2526±30	33985±544
3.	Kantarion, cela biljka	2947±47	2304±50	1647± 28	1188±30	9683±106
4.	Pitoma nana, list	14833±237	3706±82	5927±154	1691±17	20261±182
5.	Rastavić, cela biljka	15477±248	2476±54	6464±123	1279±2,56	26954±216
6.	Kopriva, koren	7603±122	3890±86	3048±55	10629±85	19068±400
7.	Kopriva, list	35399±566	6264±138	7578±68	932±15	32713±164
8.	Majkina dušica, cela biljka	8076±129	1876±41	2339±86	1191±14	16672±11
9.	Kukuruz, svila	2738±43	4680±103	1909±29	2249±14	25045±225
10.	Hibiskus, cvet	11099±178	2209±49	4332±30	5161±26	25865±284
11.	Beli slez, koren	4031±64	3273±72	3946±35	2197±6,60	10541±126
12.	Kamilica, cvet	9645±154	5975±132	3588±47	2461±30	26681±374
13.	Šipak, plod	6401±102	1545±34	2394±17	3033±6,07	16546±248
14.	Vrijesak, cela biljka	21088±337	1949±43	5566±61	1804±20	18711±224
15.	Kantarion, cela biljka	6002±96	4207±93	2470±42	1188±37	14824±430
16.	Divlja nana, cela biljka	10794±173	5092±112	1936±54	688±8	22540±383

apsorpciju preko korenog sistema. Iz zemljišta se apsorbuju u jonskoj formi: Ca^{2+}, $H_2PO_4^-$, HPO_4^{2-}, Mg^{2+}, K^+ i Na^+, pri čemu dolaze do izražaja mehanizmi kompeticije i antagonizama među različitim jonima [17,18]. Sadržaji Ca, P, Mg, K i Na u našim zemljištima variraju. Treba istaći da se unošenjem veštačkih đubriva, značajno utiče na njihovu koncentraciju u zemljištu. Maksimalne vrednosti sadržaja za Ca i P od 35399, odnosno 6264 mg/kg, izmerili smo u čaju od lista koprive, a maksimalnu vrednost za Na od 10629 mg/kg u korenu koprive. Maksimalne vrednosti za K i Mg od 33985, odnosno 7816 mg/kg, izmerene su u čaju od bosiljka (cela biljka). Treba posebno istaći, visoki sadržaj K u skoro svim analiziranim uzorcima biljnih čajeva, što ukazuje na primenu veštačkih đubriva i na njegovu bioraspoloživost u datim zemljištima. Kalijum se usvaja za sve vreme rasta biljaka, nešto intenzivnije u početku, a usporava se pri završetku vegetacije. Sadržaj K u našim zemljištima varira od 0,5–3% [17]. Kalijum u svim analiziranim uzorcima pokazuje najmanje varijacije, dok natrijum ima najveće varijacije usled velikog raspona između maksimalne (10629 mg/kg) i minimalne vrednosti sadržaja (293 mg/kg) u uzorcima biljnih čajeva. To je posledica velike pokretljivosti Na u zemljištu i činjenice da se ovaj elemenat lako ispira iz tla. Naime zemljište ima slabu adsorptivnu moć prema natrijumu [17].

Upoređivanjem sadržaja makroelemenata u čaju pitome nane (list) i čaju divlje nane (cela biljka) sa prirodnog staništa, ističe se visoki sadržaj Mg u listu pitome nane, što se objašnjava njegovom velikom koncentracijom u sastavu hlorofila. Takođe upoređivanjem biljnih čajeva: cele biljke kantariona uzorak 3 i uzorak 15, uočava se da uzorak lekovite biljke sa prirodnog staništa (uzorak 15), sadrži višu koncentraciju makroelemenata. Posebno se ističe i viši sadržaj Ca i K, dok je sadržaj Na isti.

Ražić i saradnici u svom radu iz 2006. godine [19], analizirajući lekovito bilje i zemljište, izmerili su u listu mente (nane) i bosiljku sadržaj za Ca i Mg čije vrednosti korespondiraju sa našim rezultatima, dok su vrednosti za K niže u odnosu na naše rezultate merenja.

Kékedy-Nagy i Ionescu analiziraju 16 elemenata u 14 komercijalnih biljnih čajeva uz primenu plamene atomske emisione spektrometrije i atomske absorpcione spektrometrije [20]. Sadržaj Ca u kamilici i koprivi korespondira sa našim rezultatima, dok je u pitomoj nani sadržaj Ca koji smo mi izmerili tri puta veći. Sadržaj Mg u sva tri spomenuta biljna čaja, je u saglasnosti sa rezultatima našeg merenja. U slučaju koprive za upoređivanje sadržaja Ca i Mg uzimali smo naš uzorak kopriva (list). Sadržaj K u kamilici je dva puta manji u odnosu na naša merenja. Posebno treba istaći sadržaj Na, gde se vrednosti razlikuju za red veličine. Naime rezultati naših ispitivanja ukazuju na viši sadržaj Na u spomenutim biljnim čajevima, što se verovatno može objasniti različitim pedološkim karakteristikama zemljišta i primenom veštačkih đubriva.

Ražić i saradnici u radu iz 2005. godine određuju pored ostalih elemenata i sadržaj K, Ca i Mg u 26 uzoraka lekovitog bilja uzgajanog u Srbiji [21]. U uzorcima: pitoma nana (list), kamilica (cvet), kopriva (list), kopriva (koren), kantarion (cela biljka), beli slez (koren), hajdučka trava (cela biljka) i bosiljak (cela biljka); izmerene vrednosti za K, Ca i Mg su u saglasnosti sa rezultatima naših ispitivanja, uz manje varijacije. Pri tome, treba istaći uzorak koprive (list), gde smo mi izmerili vrednost za Ca od 35399 mg/kg, što je dva puta više u odnosu na rezultat spomenutih autora od 1,68%, tj. 16800 mg/kg. S druge strane, u uzorku kamilice (cvet), spomenuti autori su za K izmerili vrednost od 5,89% (58900 mg/kg), što je dva puta više u odnosu na naš rezultat merenja od 26681 mg/kg suve materije. Razlog verovatno leži u primeni agrotehničkih mera.

Gjorgieva i saradnici u radu mere elemente: Zn, Cu, Fe, Cr, Ca, K, Li, Mg i Na, u četiri lekovite biljke sa planine Plačkovica [22]. Maksimalne vrednosti kalcijuma i magnezijuma su izmerili u koprivi (list) i to: 23279,56±±7,59, odnosno 4296,66±1,07 mg/kg, što je manje u odnosu na naše rezultate, kada je u pitanju ovaj biljni čaj.

Maksimalne vrednosti za K i Na izmerene su u kamilici: 28173,24±1,66 i 457,59±1,02 mg/kg, redom. Sadržaj K je u skladu sa našom vrednošću kada je u pitanju kamilica, dok sadržaj Na u našem slučaju ima veću vrednost (2461±30 mg/kg). Ove razlike u sadržajima Ca, Mg i Na verovatno su uzrokovane prirodom uzoraka. U našem ispitivanju uzorci su bili uzgajane lekovite biljke.

Ražić i saradnici u radu iz 2008. godine [23] određuju pored ostalih elemenata i sadržaj K, Ca i Mg u kamilici (cvet) i hajdučkoj travi (cela biljka). Sadržaj Ca i Mg, u obe lekovite biljke, je u saglasnosti sa rezultatima našeg merenja. Međutim, rezultati naših ispitivanja ukazuju na znatno viši sadržaj K.

Na osnovu rezultata naših istraživanja, može se konstatovati da ispitivani biljni čajevi predstavljaju dobar izvor makroelemenata za čoveka.

U tabeli 4 dati su rezultati merenja toksičnih elemenata u ispitivanim čajevima.

Kadmijum, olovo, živa, arsen i antimon su fiziološki nekorisni, nepotrebni i toksični [24]. Za ovu grupu elemenata često se koristi izraz “teški metali”, koji obuhvata i esencijalne teške metale (Cu, Fe, Mn, Ni, Zn i drugi), koji su u vrlo malim količinama neophodni za živi svet. Teški metali (Pb, Cd, Cu, Cr i drugi), kao polutanti u radnoj i životnoj sredini su ozbiljan zdravstveni i ekološki problem zato što su toksični, nisu razgradivi i imaju dugo poluvreme života u zemljištu [25]. Plodna zemljišta snabdevaju biljke skoro svim hemijskim elementima, koji su im potrebni za rast. Pri tome zemljišta mogu predati biljkama i toksične elemente (Hg, Pb i Cd)

Tabela 4. Sadržaj toksičnih elemenata u uzorcima biljnih čajeva (aritmetička sredina±SD), mg/kg suve materije
Table 4. Content of toxic elements in the herbal teas samples (mean±SD), mg/kg dry matter

Red. broj	Biljka	Element				
		Cd	Hg	Pb	As	Sb
1.	Hajdučka trava, cela biljka	0,499±0,001	0,012±0,001	0,373±0,004	0,045±0,002	0,014±0,001
2.	Bosiljak, cela biljka	0,076±0,001	0,004 ±0,001	0,416±0,003	0,277±0,003	0,017±0,001
3.	Kantarion, cela biljka	0,539 ±0,002	0,006±0,001	0,207±0,001	0,031±0,001	0,010±0,001
4.	Pitoma nana, list	0,042±0,001	0,017±0,001	0,715±0,001	0,215±0,003	0,028±0,001
5.	Rastavić, cela biljka	0,048±0,001	0,001±0,001	0,323±0,002	0,544±0,003	0,010±0,001
6.	Kopriva, koren	0,030±0,001	0,014±0,001	1,048±0,004	0,253±0,003	0,068±0,001
7.	Kopriva, list	0,024±0,001	0,013±0,001	1,050±0,008	0,143±0,001	0,044±0,020
8.	Majkina dušica, list	0,257±0,001	0,009±0,001	1,340±0,006	0,270±0,001	0,020±0,001
9.	Kukuruz, svila	0,027±0,001	0,008±0,001	0,500±0,006	0,100±0,001	0,015±0,001
10.	Hibiskus, cvet	0,118±0,001	<0,001± 0,001	0,286±0,001	0,043±0,001	0,005±0,001
11.	Beli slez, koren	0,044±0,001	<0,001± 0,001	0,064±0,002	0,030±0,001	0,004±0,001
12.	Kamilica, cvet	0,146±0,001	<0,001± 0,001	0,090±0,001	0,038±0,001	0,010±0,001
13.	Šipak, plod	0,014±0,001	<0,001± 0,001	0,292± 0,001	0,071± 0,002	0,007± 0,001
14.	Vrijesak, cela biljka	0,020±0,001	0,011±0,001	0,358±0,001	0,118±0,001	0,015±0,001
15.	Kantarion, cela biljka	0,645±0,003	<0,001± 0,001	0,354±0,002	0,109±0,004	0,012±0,001
16.	Divlja nana, cela biljka	0,026±0,001	<0,001± 0,001	0,459±0,003	0,060±0,003	0,015±0,001

ili pak visoke koncentracije drugih elemenata koje mogu biti toksične (Cu, Co, Cr, Ni i drugi) [26]. Dodatni izvori kontaminacije biljaka teškim metalima su atmosferske padavine, zaštitna hemijska sredstva, đubriva, industrijska postrojenja, termoelektrane, blizina rudnika i frekventnih saobraćajnica.

Kadmijum, je već u vrlo niskim koncentracijama veoma otrovan za žive organizme, a smatra se da ima i kancerogena svojstva [26]. Prosečan sadržaj Cd u zemljištima Vojvodine iznosi 0,63 mg/kg [27], a što je ispod zakonskog limita od 3 mg/kg [28]. Biernacka i Maluszyński iznose podatke sadržaja Cd u zemljištu Poljske, koji za nezagađene regione iznosi 1,4–3,2 mg/kg, a za regione koji su pod jakim antropološkim uticajem sadržaj iznosi 4,6–64,0 mg/kg suvog zemljišta [29]. Kadmijum usvojen iz hranljive podloge uglavnom se zadržava u korenu biljke. Joni Ca^{2+} i Zn^{2+} inhibiraju usvajanje kadmijuma. Prosečna koncentracija Cd u razvijenim listovima biljaka iznosi 0,05–0,2 mg/kg [30]. Akumulacija Cd u biljkama može da dovede do njihovog usporenog rasta, oštećenja korenog sistema, hloroze lišća i pojave crvenomrkih ivica na lišću [26]. Veliku opasnost predstavljaju gljive, koje mogu da sakupljaju Cd u visokim koncentracijama. Tako šampinjoni mogu da akumuliraju i do 6 mg Cd/kg [11]. Osnovni izvori zagađivanja zemljišta kadmijumom su: atmosferska depozicija, industrijski i komunalni otpad, fosfatna đubriva, kanalizacijski mulj, rude i topionice cinka [24].

Maksimalna vrednost Cd od 0,645±0,003 mg/kg , izmerena je u uzorku broj 15 – kantarion (lokalitet II), a minimalna 0,014±0,001 mg/kg suve materije u plodu šipka (uzorak broj 13). U tri uzorka: hajdučka trava (1) i kantarion (3 i 15), je izmerena vrednost Cd veća od 0,3 mg/kg, koja je predložena kao limit za suvi biljni materijal od strane Svetske zdravstvene organizacije-WHO [31]. Izmerene vrednosti Cd u uzorcima hajdučka trava (1), kantarion (3) i kantarion (15) su: 0,499±0,001; 0,539±0,002 i 0,645±0,003 mg/kg, respektivno. Uzorak divlje nane (16) sa lokaliteta II, sadrži 0,026±0,001 mg/kg Cd, te na osnovu toga možemo zaključiti da se ne radi o atmosferskom depozitu ili sastavu zemljišta, kao uzroku povišenog sadržaja kadmijuma u uzorku kantariona sa istog lokaliteta (15), već da je verovatno došlo do kontaminacije pri branju ili transportu dotičnog biljnog čaja. U slučaju druga dva uzorka: hajdučka trava (1) i kantarion sa tržišta Novog Sada (3), povišeni sadržaj Cd se može objasniti primenom fosfatnih đubriva pri gajenju biljnih čajeva [24]. Ražić i Kuntić su ispitivali sadržaj Cd u uzorcima nane, kamilice i šipka. Vrednosti sadržaja Cd u uzorcima nane (orig. pakovanje) i rasutom uzorku su: 0,098; 0,076 i 0,040 mg/kg redom [10], što je u saglasnosti sa rezultatima naših ispitivanja, uz manja odstupanja: 0,042±0,001 (orig. pakovanje) i 0,026±0,001 mg/kg (divlja nana-16). Izmerene vrednosti Cd u uzorcima kamilice od 0,132 (orig. pakovanje) i 0,179 mg/kg (rasuto stanje), su u saglasnosti sa rezultatom naših ispitivanja (0,146±0,001 mg/kg). Izmerene vrednosti Cd u uzorcima šipka (orig. pakovanje) i u rasutom stanju od 0,030 i 0,054 mg/kg, redom, su nešto više u odnosu na našu izmerenu vrednost (0,014±0,001 mg/kg). Ražić i saradnici [23] su izmerili sadržaj Cd u uzorku hajdučke trave od 0,24 mg/kg, što je dva puta manje u odnosu na našu vrednost (0,499 mg/kg). Gentscheva i saradnici su u uzorcima kamilice i hibiskusa, izmerili vrednosti za sadržaj

Cd: 0,45±0,02 i 0,14±0,01 mg/kg, redom [32]. Izmerena vrednost za Cd u hibiskusu, je u saglasnosti sa rezultatom naših ispitivanja (0,118±0,001 mg/kg), dok vrednosti u kamilici odstupaju. U ovom merenju spomenuti autori koriste metodu elektrotermalne atomske apsorpcione spektrometrije (ETAAS).

Živa (Hg), je jedan od najjačih otrova među teškim metalima. U organskim jedinjenjima ima izražena mutagena i teratogena svojstva [26]. Osnovni mineral Hg u prirodi je cinobarit-HgS. Značajan izvor žive u površinskim slojevima zemljišta je atmosferski depozit, koji sa ljudskom aktivnošću postaje sve značajniji. Upotrebom mineralnih đubriva, otpadnih muljeva, kreča i fungicida, povećava se koncentracija Hg u zemljištu. Živa se unosi u atmosferu sagorevanjem fosilnog goriva, preko vulkanskih erupcija i industrijskih procesa. Uobičajena vrednost sadržaja Hg u zemljištima kreće se u opsegu 0,03–0,06 mg/kg [33]. Sadržaj Hg u ispitivanim zemljištima Vojvodine je varirao od 0,010–0,054 ppm [17]. Novijim studijama, Hg u uzorcima zemljišta u Vojvodini nije utvrđena [27]. Biljke lako apsorbuju živu iz hranljivih rastvora, koja se zatim prenosi na celu biljku [11]. Živa narušava građu biomembrana i menja aktivnost enzima, čime narušava promet materije i inhibira rast i razviće biljaka [34]. Maksimalno dozvoljen sadržaj Hg u zemljištu Republike Srbije je 2 mg/kg zemlje [28]. Maksimalna vrednost od 0,017±0,001 mg/kg je izmerena u uzorku pitome nane-list (4), a minimalna od <0,001± ±0,001 mg/kg u uzorcima: hibiskus (10), beli slez (11), kamilica (12), šipak (13), kantarion (15) i divlja nana-cela biljka (16). Kowalski i Kucharski, 2007, u svom radu iznose rešenje Ministarstva zdravlja u Poljskoj, u kojem dozvoljena količina Hg u čaju iznosi 0,02 mg/kg [35]. Nacionalna regulativa u Kanadi, u sirovom lekovitom bilju i konačnim biljnim proizvodima, za sadržaj žive propisuje limite od 0,2 ppm i 0,02 mg/dan, redom [36]. Na osnovu iznetog, može se konstatovati da su vrednosti sadržaja Hg u analiziranim uzorcima biljnih čajeva manje u odnosu na gore spomenute literaturne vrednosti. Vrednosti sadržaja Hg u uzorcima nane (0,010 i 0,014 mg/kg); kamilice (0,001 i 0,025 mg/kg); i šipka (nije detektovano), koje su izmerili Ražić i Kuntić [10], u saglasnosti su sa našim rezultatima. Kowalski i Kucharski, ispitivali su sadržaj Hg u 55-različitih biljnih proizvoda. Između ostalog, izmerili su sadržaj Hg u kukuruznoj svili, nani (list), koprivi (list), kantarionu (cela biljka), hajdučkoj travi (cela biljka) i majkinoj dušici (cela biljka) [35]. Izmerene vrednosti u kukuruznoj svili (0,0040±0,0001 mg/kg); nani-list (0,0136±0,0010 mg/kg); koprivi-list (0,0096±0,0008 i 0,0109±0,0005 mg/kg); kantarionu-cela biljka (0,0054±0,0003 mg/kg), hajdučkoj travi-cela biljka (0,0077±0,0011 mg/kg) i majkinoj dušici-cela biljka (0,0061±0,0006 mg/kg), su u saglasnosti sa rezultatima naših ispitivanja.

Olovo (Pb), je otrovno za ljude i životinje, a u većim koncentracijama ometa rast biljaka. Olovo se smatra jednim od glavnih zagađivača životne sredine. U prirodi uglavnom dolazi u formi sulfida, kao mineral galenit (PbS). Prosečan sadržaj Pb u zemljištima Vojvodine iznosi 17,17 [17], odnosno 34,6 mg/kg [27]. Dozvoljena količina Pb u zemljištu, određena nacionalnom regulativom iznosi 100 ppm [28]. Biernacka i Maluszyński iznose vrednosti olova u zemljištima Poljske. Sadržaj Pb u nezagađenim zemljištima se kretao od 40–124 mg/kg suve zemlje [29]. Izvori olova u zemljištu, pa prema tome i u biljkama mogu biti atmosferski depozit, izduvni gasovi vozila, otpadni muljevi, rudnici i topionice. Biljke usvajaju Pb iz zemljišta, vode i atmosferskih taloga, i to u vidu Pb^{2+} ili organskih jedinjenja (Pb-tetraetil i drugo). Mehanizam usvajanja ovog elementa nije još uvek dovoljno poznat. U većim koncentracijama inhibira izduživanje korena, rast lisne površine i intenzitet fotosinteze [34]. Sadržaj olova u ispitivanim uzorcima biljnih čajeva kretao se u intervalu od 0,064±0,002 (min.) do 1,340±0,006 mg/kg (maks.). Maksimalna vrednost je izmerena u uzorku majkine dušice-cela biljka (8), a minimalna u uzorku belog sleza-koren (11). Nešto veći sadržaji Pb su izmereni u uzorcima: broj 4, pitoma nana-list (0,715±0,001 mg/kg); broj 6, kopriva-koren (1,048±0,004 mg/kg) i broj 7, kopriva-list (1,050± ±0,008 mg/kg). Na osnovu vrednosti izmerenog Pb, a imajući u vidu maksimalno dozvoljenu vrednost (MDV) od 10 mg Pb/kg u biljnom materijalu, koji je propisan od strane WHO, možemo zaključiti da je sadržaj Pb u ispitivanim čajevima ispod MDV [31]. Gentscheva i saradnici mere sadržaj olova u uzorcima kamilice i hibiskusa [32]. Izmerene vrednosti Pb: 1,40±0,05 (kamilica) i 0,78±0,05 mg/kg (hibiskus) se razlikuju od rezultata dobijenih u našem istraživanju. Ražić i Kuntić [10], pored ostalog, ispitivali su sadržaj Pb u uzorcima nane, kamilice i šipka. Sadržaj Pb u nani (rasuto stanje), od 0,580 mg/kg, je u saglasnosti sa rezultatom naših ispitivanja za uzorak nane sa lokaliteta II (16) od 0,459± ±0,003 mg/kg, dok se vrednosti sadržaja Pb u uzorcima kamilice i šipka značajno razlikuju. Tako, izmerena vrednost u uzorku kamilice (originalno pakovanje), od 0,856 mg/kg, se značajno razlikuje u odnosu na naš rezultat od 0,090±0,001 mg/kg. Izmerene vrednosti Pb u uzorcima čajeva su ispod vrednosti MDK koja je propisana nacionalnom zakonskom regulativom (maks. 5 mg/kg za domaći čaj) [37].

Arsen u izgradnji zemljine kore učestvuje sa 1,5 ppm [26]. Zbog velike toksičnosti, važan je element za ocenu kvaliteta životne sredine. U prirodi dolazi u formi sulfida As_4S_4 i As_2S_3. Njegovo ponašanje u zemljištu slično je fosforu [17]. Pokretljivost i fitotoksičnost As je veća u peskovitim u odnosu na glinena zemljišta [38]. Sadržaj arsena u poljoprivrednim zemljištima se kreće u opsegu od 0,1–50 mg/kg, a uobičajena vrednost je 1–20 mg/kg [33]. Prosečna vrednost As u zemljištu Vojvodine iznosi 2,19 mg/kg [17], ili pak nije utvrđen [27]. Maksimalno dozvoljena koncentracija za As u zemljištu Srbije je do

25 mg/kg zemlje [28]. Izvori As u zemljištu, pa prema tome i u biljkama su herbicidi, fosforna đubriva, atmosferski depozit, topionice bakra i otpadni muljevi. Sadržaj arsena u biljkama je obično znatno niži nego u zemljištu i nije neophodan za biljke. Jedan broj autora ukazuje na stimulativno delovanje niskih koncentracija As na rast korena. Međutim, visoka koncentracija As ima negativan efekat [34]. Normalna koncentracija As u razvijenim listovima različitih biljnih vrsta iznosi 1-1,7 ppm/po suvoj masi, dok se vrednosti od 5-20 ppm As smatraju toksičnim [30]. Karak i Bhagat u svojoj reviji [39] iznose podatak da je koncentracija As u svežem lišću i mladim izdancima čaja (poreklo Kina): 0,024–0,066, odnosno 0,021–0,073 mg/kg.

Maksimalna vrednost od 0,544±0,003 mg/kg je izmerena u uzorku: rastavić-cela biljka (5), a minimalna od 0,030±0,001 mg/kg u uzorku belog sleza-koren (11). Takođe, niske vrednosti su dobijene u uzorcima: hibiskus-cvet (10); kamilica-cvet (12); šipak-plod (13) i divlja nana -cela biljka (16). Sadržaj As u uzorcima čajeva je ispod vrednosti od 1 mg/kg , koji propisuje nacionalna zakonska regulativa [37]. Ražić i Kuntić su, između ostalog, ispitivali i sadržaj As u uzorcima nane, kamilice i šipka [10]. Vrednosti sadržaja As u uzorku nane (orig. pakovanje) od 0,086 mg/kg i uzorku (rasuto stanje) od 0,155 mg/kg, su u saglasnosti sa našim rezultatima od 0,060±0,003 mg/kg, divlja nana-cela biljka (16) i 0,215±±0,003 mg/kg, pitoma nana-list (4). Takođe, sadržaj As u uzorcima ploda šipka od 0,101 mg/kg (orig. pakovanje) i 0,088 mg/kg (uzorak u rasutom stanju), korespondira sa našim rezultatom (0,071±0,002 mg/kg). Međutim, rezultati dobijeni za uzorak kamilice se razlikuju za red veličine.

Szentmihályi i saradnici [40] su izmerili sadržaj As u koprivi (herba) od 3,63±1,60 mg/kg, što se znatno razlikuje u odnosu na rezultat naših ispitivanja.

Antimon, je veoma malo zastupljen u zemljinoj kori. Gradi mineral stibnit-Sb_2S_3 i druge sulfide. Smatra se da je toksičan element, ali pošto su njegova jedinjenja uglavnom nerastvorna, obično ne predstavlja ozbiljniji problem za životnu sredinu [26]. Sadržaj Sb u poljoprivrednim zemljištima varira u intervalu od 0,05–260 mg/kg, a uobičajena vrednost je 2 mg/kg [33]. Normalne koncentracije Sb u razvijenom lišću različitih biljnih vrsta iznose 7–50 mg/kg suve mase, dok se vrednost od 150 mg/kg smatra toksičnom [30]. Izvor zagađenja životne sredine antimonom su otpadne vode pri postupcima dobijanja stakla i metala [41]. Zemljišta u industrijskim područjima mogu biti veoma obogaćena antimonom [26].

Maksimalna vrednost je izmerena u uzorku koprive-koren (16) od 0,068±0,001 mg/kg, a minimalna od 0,004±0,001 mg/kg u belom slezu-koren (11). Interesantno je primetiti da, uzorak kantariona-cela biljka(3) nabavljen na tržištu Novog Sada ima skoro isti sadržaj Sb kao i uzorak kantariona-cela biljka (15) sa prirodnog staništa: 0,010±0,001, odnosno 0,012±0,001 mg/kg. Nookabkaew i saradnici su, pored ostalog, ispitivali sadržaj Sb u uzorcima čaja – *Camellia sinensis*. Vrednost antimona se kretala u opsegu od 0,002–0,076 mg/kg suve materije uz prosečnu vrednost od 0,022 mg Sb/kg suve materije. Ako ovo uporedimo sa prosečnom vrednošću sadržaja Sb u našim uzorcima biljnih čajeva, vidimo da su se vrednosti skoro podudarile, iako se radi o različitim lekovitim biljkama i geografskim područjima [42].

U tabeli 5 data je matrica Pirsonovih koeficijenata korelacije sadržaja toksičnih elemenata.

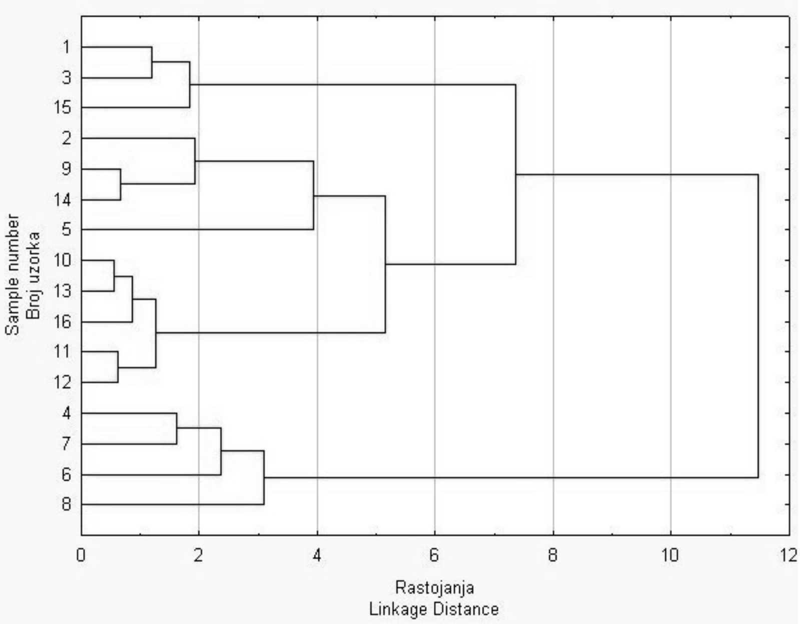
Iz tabele 5 je vidljiva visoka korelacija u sadržaju između olova i žive, antimona i žive i antimona i olova. Grupisanje biljnih čajeva na osnovu sadržaja toksičnih elemenata, izvršeno je na osnovi Ward metode hijerarhijske klaster analize, primenjene na standardizovane podatke, na osnovu Euklidskog odstojanja, a vizualizacija je data pomoću dendrograma (slika 1). Primenom metode glavnih komponenata (PCA – *Principal component analysis*), utvrđeno je da u objašnjavanju polaznog skupa promenjivih, dve glavne komponente učestvuju sa 73,7%. U prvoj komponenti (PC1), dominiraju Hg, Pb i Sb, a u drugoj komponenti (PC2), dominiraju Cd i As (Tabela 6).

Izdvojene glavne komponente čine osnovu za formiranje 3 klastera (slika 2), a što je u saglasnosti sa gore prikazanim dendrogramom (slika 1). Prvi klaster čine uzorci hajdučka trava (1), kantarion (3 i 15) u kojima je prisutan veći sadržaj Cd. Drugi klaster čine uzorci kopriva-koren (6), kopriva-list (7), pitoma-nana list (4) i majkina dušica-cela biljka (8) za koje je karakterističan veći sadržaj Hg, Pb i Sb. Treći klaster obuhvata ostale čajeve (slika 2).

Tabela 5. Matrica Pirsonovih koeficijenata korelacije sadržaja toksičnih elemenata
Table 5. Matrix of Pearsons correlation coefficients of toxic elements contents

	Cd	Hg	Pb	As	Sb
Cd	1,0000				
Hg	–0,0761	1,0000			
Pb	–0,1454	0,6448[a]	1,0000		
As	–0,2515	0,1276	0,3820	1,0000	
Sb	–0,2362	0,6917[a]	0,7354[a]	0,2828	1,0000

[a]Visoka statističku značajnost, $p < 0,01$



Slika 1. Klaster analiza zasnovana na standardizovanim podacima (Ward metoda, Euklidska rastojanja), koja se odnosi na toksične elemente.
Figure 1. Cluster analysis based on standardized data (Ward`s method, Euclidean distances), using toxic elements.

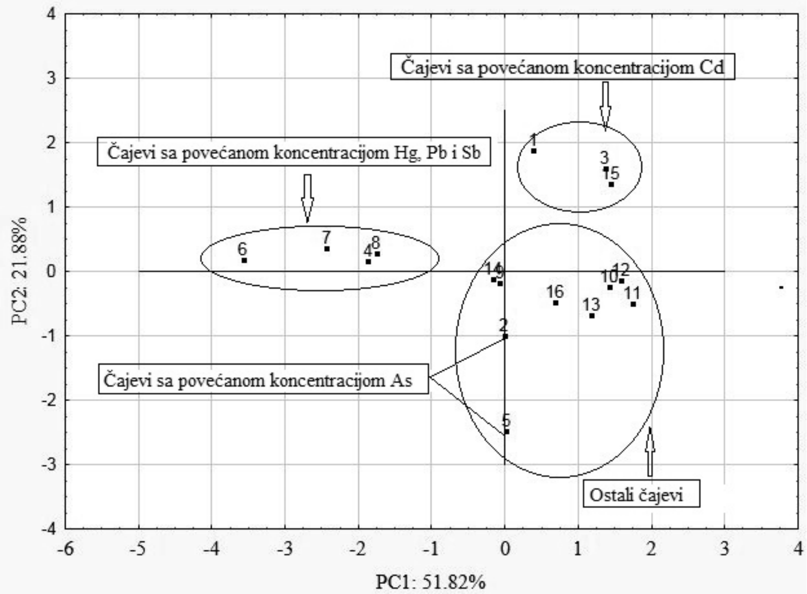
Tabela 6. Korelacije izdvojenih glavnih komponenata sa polaznim promenjivim
Table 6. Correlation of principal components extracted from the initial variables

Element	PC1	PC2
Cd	0,330304	**0,749879**
Hg	**–0,805139**	0,387223
Pb	**–0,888594**	0,121070
As	–0,489588	**–0,591601**
Sb	**–0,896836**	0,131549

Na slici 2 data je projekcija slučajeva (čajeva) na prve dve glavne komponente.

ZAKLJUČAK

Šesnaest uzoraka od 14 različitih vrsta biljnih čajeva, čije je konzumiranje značajno među stanovništvom Srbije, analizirano je na sadržaj makroelemenata i toksičnih elemenata, sa ciljem da se dobije uvid u mineralni status, a samim time i u bezbednost lekovitih



Slika 2. Projekcija slučajeva (čajeva) na prve dve glavne komponente.
Figure 2. Showing of cases (teas) on two first principal components.

biljaka, koji služe za spravljanje popularnih čajnih napitaka. Sadržaj analiziranih elemenata nalazi se unutar intervala vrednosti koje se navode u literaturnim izvorima, uz veće ili manje varijacije, a kao rezultat različitih uticaja kao što su: vrsta biljaka, primena agrotehničkih mera i pedološke karakteristike zemljišta. Analiza je i ovom prilikom ukazala na najveći sadržaj makroelemenata (Ca, P, Mg, Na i K) u lekovitim biljkama i to na prvom mestu kalcijuma i kalijuma. Maksimalna vrednost je izmerena za kalijum u uzorku bosiljka-2 (33985±544 mg/kg). Najveća varijacija u sadržaju je utvrđena za natrijum, a najmanja kod kalijuma. Izmerene vrednosti ukazuju na to, da biljni čajevi mogu poslužiti kao blagodatni izvor makroelemenata u ljudskoj ishrani. Izmerene vrednosti sadržaja toksičnih elemenata (Hg, Cd, As, Pb i Sb), su niske, što je bilo očekivano i što je dobro sa stanovišta bezbednosti biljnih čajeva. Izmerene maksimalne vrednosti su: Cd (0,645±0,003 mg/kg); Hg (0,017±0,001 mg/kg); Pb (1,340±0,006 mg/kg); As (0,544±0,003 mg/kg) i Sb (0,068±0,001 mg/kg). Treba istaći tri uzorka: hajdučka trava (1), kantarion (3) sa tržišta Novog Sada i kantarion (15) sa prirodnog staništa u kojima je izmerena vrednost Cd (0,499±0,001; 0,539±0,002 i 0,645±0,003 mg/kg, redom) veća od 0,3 mg/kg suve materije, a koja predstavlja preporučeni dozvoljeni gornji nivo Cd u osušenom biljnom materijalu. Izračunavanjem koeficijenata korelacije, utvrđena je visoka statistička značajnost ($p < 0,01$) između sadržaja olova, antimona i žive. Primenom Ward metode hijerarhijske klaster analize, primenjene na standardizovane podatke sadržaja toksičnih elemenata, a na osnovu Euklidskog odstojanja, dobivena su tri klastera. I-klaster čine uzorci hajdučka trava (1), kantarion (3 i 15), u kojima je prisutan veći sadržaj Cd. II-klaster čine uzorci kopriva-koren (6), kopriva-list (7), pitoma nana-list (4) i majkina dušica-cela biljka (8) za koje je karakterističan veći sadržaj Hg, Pb i Sb, a III-klaster obuhvata ostale čajeve. Danas su na tržištu prisutni različiti proizvodi, među kojima ima i onih koji su zdravstveno neispravni po određenim parametrima i koji ostavljaju negativne uticaje na zdravlje i radnu sposobnost čoveka. Upravo zbog toga, potrebna je stalna briga i monitoring nadležnih institucija nad svim proizvodima koji se unose u ljudski organizam. U vezi s tim, nameće se i potreba za što pouzdanijim i bržim metodama kontrole kvaliteta lekovitog bilja, pri čemu posebno treba obratiti pažnju na sadržaj toksičnih elemenata.

Zahvalnica

Istraživanje je finansirano od strane Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije, broj projekta TR31071.

LITERATURA

[1] R. Kastori, I. Maksimović, Ishrana biljaka, Vojvođanska akademija nauka i umetnosti, Novi Sad, 2008.

[2] R. Kastori, Fiziologija biljaka, Feljton, Novi Sad, 1998.

[3] R. Kastori, N. Petrović, I. Maksimović-Arsenijević, Teški metali i biljke, u: R. Kastori (ed.), Teški metali u životnoj sredini, Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 1997, str. 197–257.

[4] Č. Obračević, Osnovi ishrane domaćih životinja, Naučna knjiga, Beograd, 1990.

[5] M. Živkov-Baloš, Ž. Mihaljev, Z. Mašić, Količine makro i mikroelemenata u hranivima sa područja Vojvodine, Savrem. Poljopr. **48** (1999) 285–288.

[6] S. Maksimović, M. Jakovljević, D. Radanović, Uticaj agroekoloških faktora na hemijski sastav žalfije (*Salvia officinalis L.*), Knjiga apstrakata, V-Manifestacija, Dani lekovitog bilja, Banja Koviljača, 1998, 14-19 septembar, str. 28–29.

[7] D. Radanović, S. Antić-Mladenović, T. Nastovski, Influence of soil characteristics and nutrient supply on medicinal and aromatic plants. Proceedings from the Third Conference on Medicinal and Aromatic Plants of Southeast European Countries, Belgrade, Serbia, 2006, pp. 20–28.

[8] S. Seenivasan, N. Manikandan, N.N. Muraleedharan, R. Selvasundaram, Heavy metal content of black teas from south India, Food Control. **19** (2008) 746–749.

[9] N. Kovačević, Osnovi farmakognozije, Srpska školska knjiga, Farmaceutski fakultet, Beograd, 2002.

[10] S. Ražić, V. Kuntić, Diverse elements in herbal tea products consumed in Serbia using inductively coupled plasma mass spectrometry, Int. J. Food Prop. **16** (2013) 1–8.

[11] I.J. Vučetić, T.Lj. Krstić, Mineralne supstance u ishrani i njihov biološki značaj, Velarta, Beograd, 2000.

[12] Lj. Trajković-Pavlović, I. Gajić, M. Pecelj-Gec, Preporučeni dnevni unos hranljivih materija, vol. 2, Mineralni sastojci. Savezni zavod za zaštitu i unapređenje zdravlja, Beograd, 1996.

[13] D. Kostić, S. Mitić, A. Zarubica, M. Mitić, J. Veličković, S. Ranđelović, Content of trace metals in medicinal plants and their extracts, Hem. Ind. **65** (2011) 165–170.

[14] N. Kovačević, R. Jančić, 100 Lekovitih biljaka, kroz tradiciju i savremeni život srpskog naroda, Srpska školska knjiga, Beograd, 2003.

[15] Milestone's Tips & Techniques Book and Aplication - An Operations Overview & Practical Guide by Keneth Borowsky, Method: Tea Leaves - Application Note 003, 2000.

[16] F.T. Cox, An Introduction to Multivariate Data Analysis, Oxford University Press Inc., Oxford, 2005.

[17] M. Ubavić, D. Bogdanović, Agrohemija, Univerzitet u Novom Sadu, Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, 2001.

[18] D. Bogdanović, M. Ubavić, D. Dozet, Hemijska svojstva i obezbeđenost zemljišta Vojvodine neophodnim makroelementima u: R. Kastori (ur.), Teški metali i pesticidi u

zemljištvu, Zbornik, Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, Novi Sad, 1993, pp. 197–215.

[19] S. Ražić, S. Đogo, L. Slavković, Inorganic analysis of herbal drugs. Part II. Plant and soil analysis-diverse bioavailability and uptake of essential and toxic elements, J. Serb. Chem. Soc. **71** (2006) 1095–1105.

[20] L. Kékedy-Nagy, A. Ionescu, Characterization and classification of tea herbs based on their metal content. Acta Universitatis Sapientiae, Agriculture and Environment **1** (2009) 11–19.

[21] S. Ražić, A. Onjia, S. Đogo, L. Slavković, A. Popović, Determination of metal content in some herbal drugs-Empirical and chemometric approach, Talanta **67** (2005) 233–239.

[22] D. Gjorgieva, T. Kadifkova-Panovska, K. Bačeva, T. Stafilov, Metalic Trace Elements in Medicinal Plants from Macedonia, Middle East J. Sci. Res. **7** (2011) 109–114.

[23] S. Ražić, S. Đogo, L. Slavković, Investigation on bioavailability of some essential and toxic elements in medicinal herbs, J. Nat. Med. **62** (2008) 340–344.

[24] Z. Lončarić, I. Kádár, Z. Jurković, V. Kovačević, B. Popović, K. Karalić, Teški metali od polja do stola (Heavy metals from farm to fork), Zbornik radova, 47. hrvatski i 7. međunarodni simpozij agronoma, Opatija, Hrvatska, 2012, str. 14–23.

[25] S.R. Nikolić, M.J. Jovanović, M.G. Kocić, P.T. Cvetković, R.S. Stojanović, D.T. Anđelković, S.N. Krstić, Praćenje efekata izloženosti olovu i kadmijumu u radnoj i životnoj sredini preko parametara standardne biohemijske analize krvi i aktivnosti endonukleaza jetre, Hem. Ind. **65** (2011) 403–409.

[26] L. Kolomejceva-Jovanović, Hemija i zaštita životne sredine, Ekološka hemija, Savez inženjera i tehničara Srbije, Beograd, 2010.

[27] M. Čuvardić, P. Sekulić, Ž. Mihaljev, M. Živkov-Baloš, Ž. Ćupić, Essential and Toxic Elements in Soils, Feed and Food in Vojvodina Province, in Proceedings of the International Symposium on Trace Elements in the Food Chain, Budapest, Hungary, 2006, pp. 220–224.

[28] Pravilnik o dozvoljenim količinama opasnih i štetnih materija u zemljištu i vodi za navodnjavanje i metodama njihovog ispitivanja. Službeni glasnik RS, br. 23/1994.

[29] E. Biernacka, J.M. Maluszyński, The content of cadmium, lead and selenium in soils from selected sites in Poland, Polish J. Environ. Stud. **15** (2006) 7–9.

[30] A. Kabata-Pendias, H. Pendias, Trace elements in soils and plants. Third Edition, CRC PRESS, Boca Raton, FL, 2001.

[31] WHO Quality control methods for medicinal plant materials. World Health Organization, Geneva, 1998.

[32] D.G. Gentscheva, T. Stafilov, H.E. Ivanova, Determination of some essential and toxic elements in herbs from Bulgaria and Macedonia using atomic spectrometry, Eurasian J. Anal. Chem. **5** (2010) 104–111.

[33] B.J. Alloway, Heavy Metals in Soils. Blackie, Glasgow and London, Halsted Press, John Wiley & Sons, Inc. New York, 1990.

[34] R. Kastori, N. Petrović, Uticaj teških metala na biljke. Teški metali i pesticidi u zemljištu, Zbornik, Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, Novi Sad, 1993, str. 55–72.

[35] R. Kowalski, A. Kucharski, Monitoring of total mercury level in selected herbal products, Herba. Pol. **53** (2007) 236–240.

[36] World Health Organization, WHO guidelines for assessing quality of the herbal medicines with reference to contaminants and residues, 2007.

[37] Pravilnik o dopuni Pravilnika o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja, Službeni glasnik RS, br. 28/2011.

[38] D. Bogdanović, M. Ubavić, V. Hadžić, Teški metali u zemljištu, u: R. Kastori (ur.), Teški metali u životnoj sredini, Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 1997, str. 97–152.

[39] T. Karak, M. R. Bhagat, Trace elements in tea leaves, made tea and tea infusion: A review, Food Res. Int. **43** (2010) 2234–2252.

[40] K. Szentmihályi, G. Marczal, M. Then, Medicinal plants in view of trace elements, Thaiszia- J. Bot. **16** (2006) 99–107.

[41] WHO Antimony in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/74, World Health Organization, Geneva, 2003.

[42] S. Nookabkaew, N. Rangkadilok, J. Satayavivad, Determination of trace elements in herbal tea products and their infusions consumed in Thailand, J. Agric. Food Chem. **54** (2006) 6939–6944.

SUMMARY

LEVELS OF MACROELEMENTS AND TOXIC ELEMENTS IN HERBAL TEAS

Željko A.Mihaljev[1], Željko. N. Ćupić[2], Milica M. Živkov-Baloš[1], Sandra M. Jakšić[1]

[1]Scientific Veterinary Institute„Novi Sad“, Novi Sad, Serbia
[2]Scientific Institute for Reproduction and Artificial Insemination of Domestic Animals „Temerin“, Temerin, Serbia

(Scientific paper)

A total of 14 diverse herbal teas were examined, including: yarrow, basil, St.John’s wort, peppermint, horsetail, nettle, thyme, corn silk, hibiscus, marshmallow, chamomile, rosehip, heather and wild mint. The samples were prepared using the method of microwave digestion, and measurements were performed by the use of inductively coupled plasma mass spectrometry (Cd, Hg, Pb, As and Sb); atomic absorption spectrometry (Mg); emission flame photometry (Ca, K and Na) and spectrophotometry (P). Intervals of variation (mg/kg) for macroelements were: Ca (2738–35399); P (1545–6264); Mg (1647–7816); Na (293–10629) and K (9683–33985), and for toxic elements: Cd (0.014–0.645); Hg (<0.001–0.017); Pb (0.064–1.340); As (0.030–0.544) and Sb (0.004–0.068). In the three samples (yarrow and two St.John’s wort samples) measured cadmium concentration was higher than the maximum level for dried herbs, recommended by WHO. Ward’s hierarchical clustering method was performed with the aim of grouping herbal teas by the amount of toxic elements.

Keywords: herbal teas • macroelements • toxic elements • ICP-MS • AAS

Investigation of fluorescence properties of cyanidin and cyanidin 3-*O*-β-glucopyranoside

Violeta P. Rakić[1], Ajda M. Ota[2], Mihaela A. Skrt[2], Milena N. Miljković[3], Danijela A. Kostić[3], Dušan T. Sokolović[4], Nataša E. Poklar Ulrih[2,5]

[1]*College of Agriculture and Food Technology, Prokuplje, Serbia*
[2]*Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[3]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Niš, Serbia*
[4]*Faculty of Medicine, University of Niš, Niš, Serbia*
[5]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CipKeBiP), Ljubljana, Slovenia*

Abstract

The absorption and fluorescence emission spectra of cyanidin and cyanidin 3-*O*-β-glucopyranoside (Cy3Glc) at pH 5.5 in aqueous solution have been studied. The most effective fluorescence excitations of cyanidin were at the UV absorption maxima at 220 and 230 nm and at higher wavelengths at 270 and 280 nm. Cyanidin exhibits fluorescence emission maxima at 310 nm and in the visible range at 615 nm. The most effective fluorescence excitation for the Cy3Glc was at 220 and at 230 nm, and at higher wavelengths at 300 and at 310 nm. The Cy3Glc has fluorescence emission spectra with the maximum at 380 nm and does not show fluorescence emission in the visible range. If compare fluorescence emission spectra of cyanidin and Cy3Glc, it can be seen that fluorescence emission intensity for cyanidin is significantly higher than that for Cy3Glc. These results revealed the impact of 3-glucosidic substitution at C-3 of aglycone (to form Cy3Glc) on the significantly decrease in fluorescence emission intensity, and disappearance of the fluorescence emission in visible wavelength range.

Keywords: anthocyanins, anthocyanidins, cyanidin, cyanidin 3-glucopyranoside, fluorescence emission spectra, UV–Vis absorption spectra.

SCIENTIFIC PAPER

UDC 547.973:543.42/.423

Hem. Ind. **69** (2) 155–163 (2015)

doi: 10.2298/HEMIND140203030R

Anthocyanins are glycosylated polyhydroxy and polymethoxy derivates of 2-phenylbenzopyrylium (flavylium) salts, where the 3-hydroxyl group is replaced by glucose or another sugar. Cyanidin 3-*O*-β-glucopyranoside (Cy3Glc) is a simple anthocyanin that is found in different berries, such as elderberries, blueberries, cowberries, whortleberries, blackcurrants, roselle and black chokeberries [1,2].

Anthocyanins have several biological activities, including antioxidant, antihepatocarcinogenic, anti-inflammatory, anti-tumor, neuroprotective, antihemolytic, anti-diabetic, hypolipidemic and cancer chemopreventive [3–17]. Epidemiological studies have suggested that anthocyanins have cardioprotective functions in human [18], and other studies have suggested that anthocyanins inhibit tumor-cell growth *in vitro* and suppress tumor growth *in vivo* [19].

These are natural, water soluble, non-toxic pigments that can show a variety of colours, from orange to blue [20–22]. Today, there is considerable interest in the development of food colorants from natural sources to replace synthetic food colorants [23,24]. The reason behind this is to develop safe, economical and efficient food colorants to replace the banned coal tar and azo dyes [23,25]. Here, the coloured anthocyanins have some advantages: they are safe, coloured especially in the red region, and relatively soluble, which simplifies their incorporation into aqueous food systems [1,23].

However, there are some limitations to the use of anthocyanins as food colorants, which include their chemical instability, their need for purification, and their tinctorial power, which is nearly 100-fold lower than that of the coal tar dyes. In food products, a number of reactions can occur, and pH can affect their colours, although the major problem associated with the use of anthocyanins as food colorants is their temperature, oxygen, light and enzymatic instability [1,21,23,26–29].

Similar to the other anthocyanidins and anthocyanins, cyanidin and Cy3Glc exist in various structural forms in aqueous environments, which depend on the pH (Figure 1). Spectral investigations have revealed the coexistence of the flavylium cation (Figure 1, AH^+), quinonoid base (Figure 1, A), two hemiacetal forms (Figure 1, B), and two chalcone forms (Figure 1, C). The equilibrium between these structures is highly pH dep-

---

Correspondence V. Rakić, College of Agriculture and Food Technology, Ćirila i Metodija 1, 18400 Prokuplje, Serbia.
E-mail: violetachem@gmail.com

Paper received: 3 February, 2014
Paper accepted: 9 April, 2014

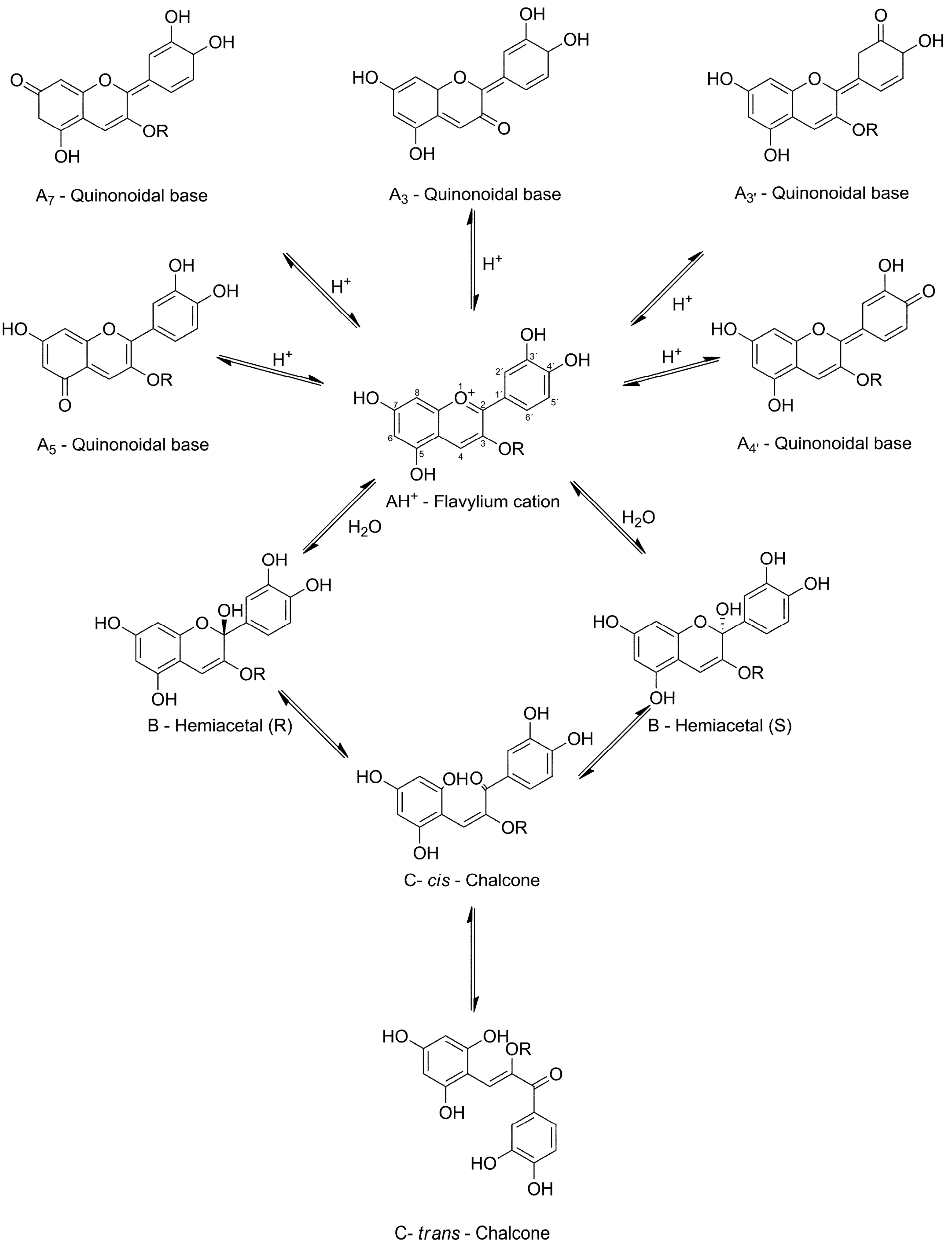


Figure 1. Transformations of cyanidin and Cy3Glc in aqueous solutions at different pH values. At C-3 for cyanidin R = H, for Cy3Glc R = Glc [23,30–32,36].

endent, and except for the *cis*-*trans* chalcone isomerisation, all of these transformations are fully reversible, with reaction half-times of several minutes or less. At pH < 2, the anthocyanins exist predominately as orange, red or purple flavylium cations, which are characterised by an extended π-electron system, and consequently show electronic absorption in the visible region [1,23,30–35].

Upon elevation of pH 4-6 kinetic and thermodynamic competition occurs between the hydratation reaction on position 2 of the flavylium cation (AH^+) and the proton transfer reactions related to the acidic hydroxyl groups of the aglycone. First reaction, hydratation *via* the nucleophilic attack, leading to colorless hemiacetal structure (B, hydration equilibrium); the latter can subsequently undergo ring-opening, forming pale yellow *cis*-chalcone (C_{cis}) with restituted large π-electron system (see Figure 1), with possibility of further (usually slow) isomerisation to the *trans*-chalcone (C_{trans}). The second reaction is the fast deprotonation of flavylium cation (AH^+) leading to tautomeric more violet quinonoid bases (A) (prototropic tautomeric equilibrium). Further deprotonation of the quinonoid bases (A) can take place at pH between 6 and 7 with the formation of purplish, resonance-stabilised quinonoid anions (A^-) [1,2,23,30].

The transformations of cyanidin and Cy3Glc which undergo in aqueous media are illustrated in Figure 1 [23,30–32,36].

Some anthocyanins show measurable fluorescence, but information on this topic in the literature is scarce. The study of Drabent and coworkers [37] with red cabbage extracts, containing mainly cyanidin derivates, has shown that the colorless compounds present in these extracts fluorescence, and that this emission is pH dependent. The fluorescence emission of anthocyanins has usually been investigated using excitation in the visible region and near UV, that is, at excitation wavelength, λ_{exc} > 270 nm [31,37–39]. Low fluorescent quantum yield, especially of colored forms of anthocyanins, is one of the reasons why it is rarely applied in analytics [31].

Previously published monographs describe fluorescence of various forms of anthocyanins in aqueous environment at various pH. In particular, it was found that chalcones (C) have fluoresce emission in the spectral range 420–450 nm (λ_{exc} 320–340 nm), while hemiacetal form (B) at 370 nm. The flavylium cation (AH^+) shows weak fluorescence emission in the spectral range 570–620 nm ($\lambda_{exc} = 520$ nm). The quinonoid base (A) as anion (A^-) has fluoresce emission spectra in the range 600–665 nm [31,37–39].

The fluorescence techniques are high sensitive and nondestructive. It can add useful information on content of anthocyanins in food and drinks. This techniques are useful tool for nondestructive monitoring of anthocyanin compounds [40,41]. In the presented study we investigated the absorption and fluorescence properties of the non-sugar, aglycone, moiety of cyanidin, and the impact of its 3-glucosidic substitution (to form Cy3Glc) on the spectral parameters. An attempt has been made to correlate the absorption and emission spectra. The fluorescence of cyanidin and Cy3Glc were excited in the UV range (210–350 nm, at every 10 nm). The low concentration of dyes indicates that anthocyanin exists as monomers in these solutions.

EXPERIMENTAL

Chemicals and reagents

The chloride salts of cyanidin (2-(3,4-dihydroxyphenyl)chromenylium-3,5,7-triol chloride, CAS Number: 528-58-5, $C_{15}H_{11}O_6Cl$, molecular weight 322.7 g/mol) and Cy3Glc ((2*S*,3*R*,4*S*,5*S*,6*R*)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol chloride, CAS Number: 7084-24-4, $C_{21}H_{21}O_{11}Cl$, molecular weight is 484.8 g/mol) were obtained from Polyphenols Laboratories AS (Sandnes, Norway). Hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Aqueous solutions were prepared from Milli-Q water (resistivity > 18 MΩ cm, Millipore, Bedford, MA, USA).

Fluorescence emission measurements of cyanidin and cyanidin 3-*O*-*β*-glucopyranoside

The chloride salts of the cyanidin and Cy3Glc were dissolved in Milli-Q water to 2×10^{-5} mol dm^{-3}. The fluorescence emission spectra of cyanidin and Cy3Glc were measured at pH 5.5. This pH is chosen according to the procedure of Drabent *et al.* [31]. The required pH was achieved by addition of hydrochloric acid or sodium hydroxide, and measured using a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerland) equipped with an InLab micro electrode (Mettler Toledo, Schwerzenbach, Switzerland). These solutions were equilibrated in the dark at 25 °C, following the procedure of Brouillard *et al.* [30,42]. The fluorescence emission spectra of the cyanidin and Cy3Glc solutions were recorded at 25.0±0.1 °C, using a Cary Eclipse fluorescence spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length fluorescence cell, with slit widths with a normal band-pass of 5 nm for both excitation and emission. The excitation wavelengths used for cyanidin and Cy3Glc were in the UV range (210–350 nm, at every 10 nm). Each spectrum was multiplied by the dilution factor.

UV–Vis spectrometry of cyanidin and cyanidin 3-*O*-*β*-glucopyranoside

The chloride salts of the cyanidin and Cy3Glc were dissolved in Milli-Q water to 2×10^{-4} mol dm^{-3}. The UV–Vis spectra of cyanidin and Cy3Glc were measured at pH 0.4 and 5.5, according to the procedure of Drabent and Figueiredo *et al.* [31,38]. At pH < 2, the anthocyanins exist as flavylium cations, which show absorption in the visible region [1,23,30–35], at pH 5.5 antocyanins exist as colorless hemiacetal structure [1,21,23,30,31,43,44]. The required pH was achieved by addition of hydrochloric acid or sodium hydroxide, and measured using a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerlend) equipped with an InLab micro electrode (Mettler Toledo, Schwerzenbach, Switzerlend). These solutions were equilibrated in the dark at 25 °C, following the procedure of Brouillard *et al.* [30,42]. The absorption spectra (190–900 nm) of the cyanidin and Cy3Glc solutions were recorded at specified pH at 25.0±0.1 °C, using a Cary 100 Bio UV–Vis spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length quartz cell, with Milli-Q water as the reference. Each spectrum had the solvent spectrum subtracted and was multiplied by the dilution factor.

RESULTS AND DISCUSSION

The absorption and fluorescence emission spectra of cyanidin and Cy3Glc at pH 5.5 in aqueous solution have been examined. The impact of the 3-glucosidic substitution at the aglycone moiety of cyanidin was examined in terms of the fluorescence emission properties. This allowed the impact of the 3-glucosidic substitution at the aglycone moiety of cyanidin to be examined in terms of the spectral properties.

The self-association of cyanidin and Cy3Glc occurs at high concentrations (>10^{-3} mol dm^{-3}) [39]. In our investigations, cyanidin and Cy3Glc were dissolved to final concentrations of 2×10^{-5} for fluorescence and 2×10^{-4} mol dm^{-3} for absorption measurements. At low concentrations cyanidin and Cy3Glc exist as monomers in the studied solutions [31].

Absorption and fluorescence properties of cyanidin

The UV–Vis absorption spectra of cyanidin in aqueous solution at pH 0.4 and 5.5 are presented in Figure 2. Fluorescence emission spectra of cyanidin in aqueous solution at pH 5.5 for different excitation wavelength are shown at Figure 3.

At pH 0.4, the absorption band in the visible spectra for cyanidin was narrow, with a visible absorption maximum ($\lambda_{\text{max-vis}}$) at 516 nm (Figure 2), which corresponds to the flavylium cation (Figure 1, AH$^+$) [1,23]. At this pH value, the flavylium cation is essentially the sole species present in solution [1]. The band corresponds to the

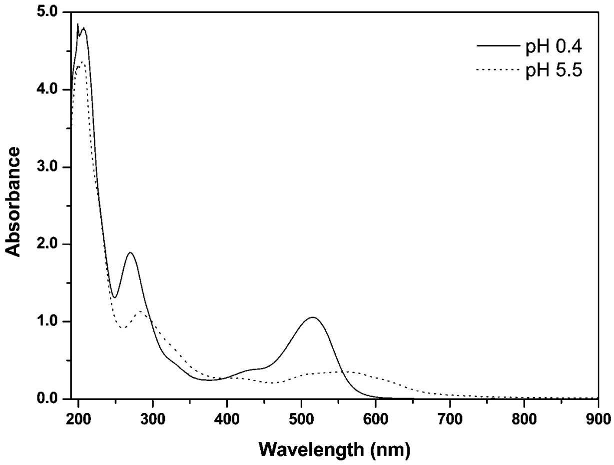


Figure 2. UV–Vis spectra of cyanidin in aqueous solution at pH 0.4 (–––) and 5.5 (·······). The concentration of cyanidin was 2×10^{-4} mol dm^{-3}, and the temperature was 25.0 ℃.

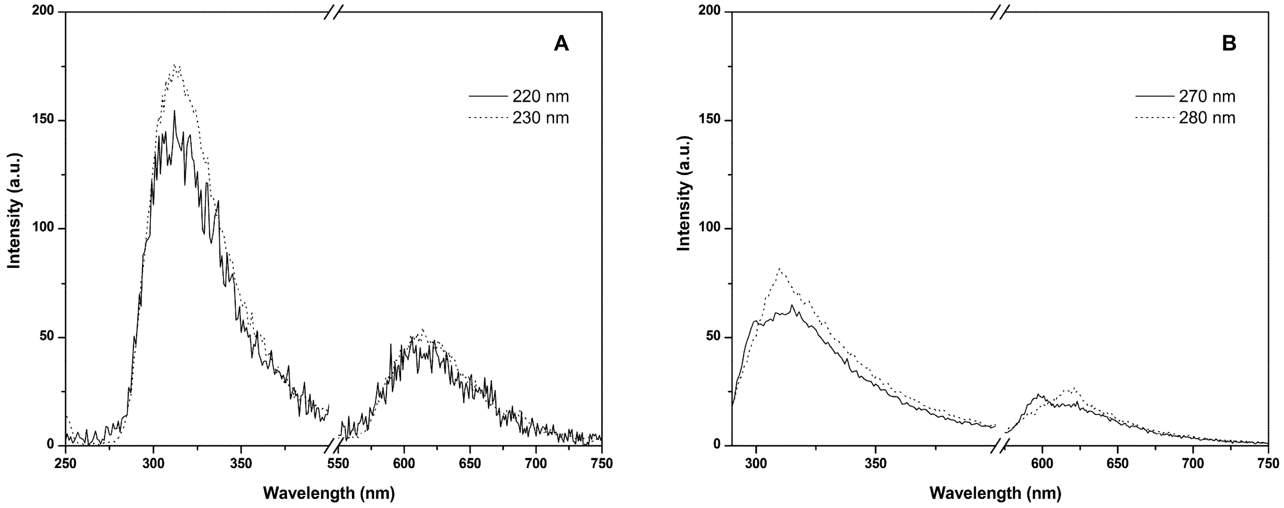


Figure 3. Fluorescence emission spectra of cyanidin in aqueous solution at pH 5.5. A) Excitation wavelength λ_{exc} = 220 (–––) and 230 (·······) nm; B) excitation wavelength λ_{exc} = 270 (–––) and 280 (·······) nm. The concentration of cyanidin was 2×10^{-5} mol dm^{-3} and the temperature was 25.0 ℃.

flavylium cation disappear at pH 5.5, whereby it completely shifts towards the colorless hemiacetal form and the coloured quinonoid bases. At the pH 5.5, the broad absorption band indicates that cyanidin is likely to be present in a few similar equilibrium structures in this pH range. There was a change in the spectral band, or a bathochromic shift, in the $\lambda_{max-vis}$ spectra of cyanidin, from 516 nm (at pH 0.4) to 560 nm (at pH 5.5). In the literature, this shift has been ascribed to the equilibrium between the flavylium cation and the quinonoid bases [1]. In the fast proton-transfer diffusion-controll-led reactions, the protons are transferred from the oxygen atoms of the C-3, C-5, C-7, C-3′ and C-4′ hydroxyl groups of the flavylium cation to a water molecule. Deprotonation occurs to some extent at all of the hydroxyl groups of the flavylium cation [1,30] (Figure 1). Both the flavylium cation and the quinonoid bases strongly absorb visible light [1,30]. The colourless hemiacetal form arises due to nucleophilic addition of water to the positively charged pyrylium ring at C-2 (Figure 1). Equilibration of this reaction occurs much slower than the proton transfer reaction, as it takes a few minutes for completion at room temperature.

Comparison of absorption spectra of cyanidin at pH 5.5 with the absorption spectra of the flavylium cation (at pH 0.4) shows that in water at pH 5.5, mainly cyanidin colourless form exists (Figure 2). Their absorption spectra in UV region differ from that of the flavylium cation especially at 270–280 nm (the range used to excite the fluorescence). At the pH of 0.4 cyanidin shows band in UV range with a λ_{max-uv} at 270 nm, while at pH 5.5 the λ_{max-uv} is at 284 nm. This absorption bands previously assigned to the hemiacetal forms indicate the expected formation of hemiacetal form on the expense of flavylium cation [1,38]. Another absorption band of very high intensity at low wavelengths exists in the UV range. The maxima of this absorption band do not change with pH and appear at around 210 nm and that is characteristic for polyphenolic compounds [45]. This wavelength is later used for fluorescence excitation. These results agree with the literature data reported for anthocyanins [33,38,46].

The excitation wavelengths for recording fluorescence emission spectra of cyanidin in aqueous solution at pH 5.5 were in the UV range (210–350 nm, at every 10 nm). According to the UV–Vis absorption spectra (Figure 2), the most effective excitation of fluorescence is measured at the first UV absorption maxima of cyanidin at 220 and at 230 nm (Figure 3A). At these wavelengths the molar absorptivities of the compound are high (ε = 14731.35 and 11581.60 $dm^3 mol^{-1} cm^{-1}$, respectively). The second less intensive UV absorption maximum of cyanidin were at around 280 nm, and accordingly, the second most effective fluorescence excitations were at 270 and 280 nm (Figure 3B), the characteristic absorption band of the flavonoids with no π-electron coupling between the two rings system (*e.g.*, hemiacetal forms) [36,38]. Obtained results are in accordance with obtained spectroscopic data (ε = = 4886.70 $dm^3 mol^{-1} cm^{-1}$ at 270 nm and 5565.1 $dm^3 mol^{-1} cm^{-1}$ at 280 nm). It was found that cyanidin has fluorescence emission in the wavelength from 280 to 700 nm with two bands, the first one with the maximum at λ_{max}^{fl} ≈ 310 nm and the second one in visible range with lower fluorescence emission intensity with maximum at λ_{max}^{fl} ≈ 615 nm (Figure 3A and B). The fluorescence emission spectral band of cyanidin in the visible range is assigned to the quinonoid bases [38].

Absorption and fluorescence emission properties of cyanidin 3-*O*-*β*-glucopyranoside

The UV–Vis absorption spectra of Cy3Glc in aqueous solution at pH 0.4 and 5.5 are presented in Figure 4. Fluorescence emission spectra of Cy3Glc in aqueous

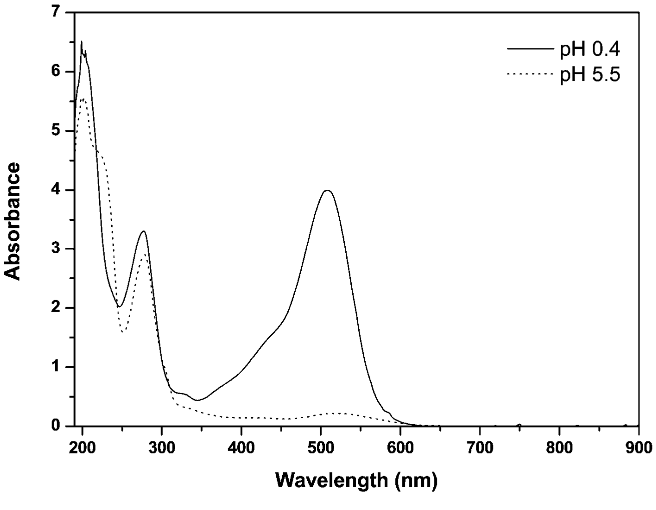


Figure 4. UV-vis spectra of Cy3Glc in aqueous solutionat pH 0.4 (–––) and 5.5 (⋯⋯). The concentration of cyanidin was 2×10^{-4} mol dm^{-3}, and the temperature was 25.0 °C.

solution at pH 5.5 for different excitation wavelength are shown at Figure 5.

Similar to cyanidin, at the lowest pH values, the absorption band in the visible spectrum for Cy3Glc was narrow with $\lambda_{\text{max-vis}}$ at 509 nm (Figure 4), and it corresponds to the flavylium cation (Figure 1, AH^+) [1,24,30]. Previously, the absorbance maximum of Cy3Glc determined in aqueous solution in the pH range from 0.8 to 3.7 was reported as 511±1 nm [2,21,24,47], which is in good agreement with our result here.

At pH 5.5, there was an extremely large decrease in the absorbance of Cy3Glc in the visible range in comparison with pH 0.4 (Figure 4). The band characteristic of the flavylium cation disappears at pH 5.5. This reflects the conversion of the coloured flavylium cation into the colourless hemiacetal form [1,23,30]. The main process in this pH range is the nucleophilic addition of water to the coloured flavylium cation. Comparison of the spectra at pH 5.5 with the absorption spectra of the flavylium cation at pH 0.4 (Figure 4) indicates that Cy3Glc exists mainly in a colourless hemiacetal and chalcone forms, formed at this pH which is in good agreement with result of Drabent *et al.* [31]. The values of molar absorptivities in visible range at 520 nm for cyanidin and Cy3Glc at pH 0.4 (ε = 5216.00 and 18544.65 $dm^3\ mol^{-1}\ cm^{-1}$, respectively) indicates the high impact of the 3-glucosidic substitution on the absorptivity of the aglycone moiety. In the pH range from 0.4 to 5.5, a bathochromic shift in the UV–Vis spectra of Cy3Glc was observed, where the visible absorption maximum is shifted from 509 to 530 nm. This indicates that the deprotonation/protonation equilibrium that occurs corresponds to the deprotonation of the small remaining amounts of the flavylium cation, into the quinonoid bases.

Beside the visible absorption maxima, Cy3Glc shows two bands in UV range with the almost constant position of absorption maxima regardless of the pH value. The first band with lower intensity has absorption maxima at 277 nm at pH 0.4 and 278 nm at pH 5.5. The maximum of the second absorption band of very high intensity and low wavelengths did not change with pH and appeared at 204 nm, characteristic for polyphenolic compounds [45]. This wavelength is later used for fluorescence excitation. These results are in good agreement with the previously published data [33,38,46].

The excitation wavelengths for recording fluorescence emission spectra of Cy3Glc in aqueous solution at pH 5.5 were in the UV range (210–350 nm, at every 10 nm). According to the UV-vis absorption spectra (Figure 4), the most effective excitation of fluorescence is measured at the first UV absorption maxima of Cy3Glc at 220 and 230 nm (Figure 5A). At this wavelengths the molar absorptivities of the compound are high (ε = 23333.00 and 21718.45 $dm^3\ mol^{-1}\ cm^{-1}$, respectively). The second less intensive UV absorption maxima of cyanidin was at around 280 nm, and accordingly, the second most effective excitation of fluorescence were at 300 and 310 nm (Figure 5B). Obtained results are in accordance with obtained spectroscopic data (ε = 5791.20 $dm^3\ mol^{-1}\ cm^{-1}$ at 300 nm and 3404.50 $dm^3\ mol^{-1}\ cm^{-1}$ at 310 nm). It was found that cyanidin has fluorescence emission spectra in the wavelength from 320 to 550 nm The fluorescence emission spectra obtained here are in accordance with results of Drabent *et al.* [31]. It was found that Cy3Glc has a fluorescence emission intensity maximum at $\lambda_{\text{max}}^{\text{fl}} \approx 380$ nm (Figure 5). The absence of the fluorescence emission band in visible range can be due to the very low concentrations of quinonoid bases, as can be seen from the absorption spectra.

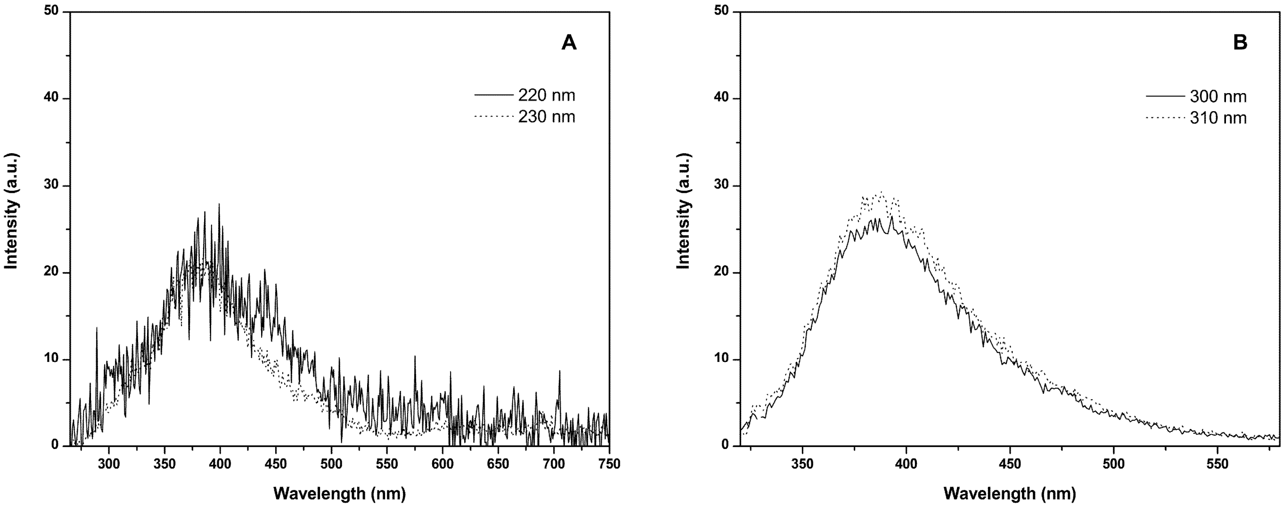


Figure 5. Fluorescence emission spectraof Cy3Glc in aqueous solution at pH 5.5. Excitation wavelength, λ_{exc}: A) 220 (–––) and 230 (·······) nm; B) 300 (–––) and 310 (·······) nm. The concentration of cyanidin was 2×10^{-5} mol dm^{-3}, and the temperature was 25.0 °C.

If compared fluorescence emission spectra (λ_{exc} was 230 nm) of cyanidin and Cy3Glc, it is seen that the fluorescence emission intensity for cyanidin is significant higher than for Cy3Glc (around 170 a.u. for cyanidin and 20 a.u for Cy3Glc) (Figures 3 and 5). Similar results were obtained by applying λ_{exc} = 270 nm. The obtained fluorescence emission intensity of cyanidin is significant higher than for Cy3Glc (around 70 a.u. for cyanidin and around 20 a.u for Cy3Glc). Our results revealed the impact of its 3-glucosidic substitution at C-3 of aglycone (to form Cy3Glc) on the significantly decrease in fluorescence emission intensity, and disappearance of the fluorescence emission band in visible wavelength range. The cyanidin glycosides are regarded as non-fluorescent or exhibit very faint fluorescence, at least in neutral and weakly acidic aqueous media [32,34,36,39,46,48]. We found that both studied compounds have fluorescence emission spectra. Best to our knowledge, this work presented here is the first fluorescence characterization of cyanidin.

CONCLUSION

The results of our work presented in this paper show that cyanidin in aqueous solution at pH 5.5 exhibits fluorescence emission spectra with the maximum of the fluorescence intensity at λ_{max}^{fl} ≈ 310 nm and surprisingly the second low intensity maxima at λ_{max}^{fl} ≈ 615 nm in the visible wavelength range. The most effective fluorescence excitation wavelengths of cyanidin were at the UV absorption maxima, the first at 220 and 230 nm and the second at the 270 and 280 nm. The fluorescence emission spectra can be obtained for Cy3Glc in aqueous solution with the maximum in the fluorescence emission at λ_{max}^{fl} ≈ 380 nm by using the excitation wavelengths 220 and 230 nm and at higher wavelengths at 300 and 310 nm. If compare fluorescence emission spectra of cyanidin and Cy3Glc, the fluorescence emission intensity for cyanidin is significantly higher than that for Cy3Glc. The Cy3Glc does not show fluorescence emission band in the visible range. These results revealed the impact of its 3-glucosidic substitution at C-3 of aglycone (to form Cy3Glc) on the significantly decrease of fluorescence emission intensity in UV range, and disappearance of the fluorescence emission band in visible range. Thus, the fluorescence spectroscopy, as a high sensitive technique, can be useful for non-destructive monitoring of anthocyanin compounds, especially in food and drinks.

Acknowledgements

The authors would like to express their gratitude for financial support to the Slovenian Research Agency through the P4-0121 Research Programme and the Bilateral Project between the Republic of Slovenia and the Republic of Serbia BI-RS/12-13-015. V.R. was partly financed by a CEEPUS SI-8402/2010 Bilateral Scholarship.

REFERENCES

[1] R. Brouillard, Chemical Structure of Anthocyanins, in: P. Markakis (Ed.), Anthocyanins As Food Color, Academic Press, New York, 1982, pp. 1–40.

[2] T. Fossen, L. Cabrita, M. Andersen, Colour and stability of pure anthocyanins influenced by pH including the alkaline region, Food Chem. **63** (1998) 435–440.

[3] A. Saija, M. Scalese, M. Lanza, D. Marzullo, F. Bonina, F. Castelli, Flavonoids as antioxidant agents: Importance of their interaction with biomembranes, Free Radic. Biol. Med. **19** (1995) 481–486.

[4] J. Azevedo, I. Fernandes, A. Faria, J. Oliveira, A. Fernandes, V. De Freitas, N. Mateus, Antioxidant properties of anthocyanidins, anthocyanidin-3-glucosides and respective portisins, Food Chem. **119** (2010) 518–523.

[5] C. Sun, Y. Zheng, Q. Chen, X. Tang, M. Jiang, J. Zhang, X. Li, K. Chen, Purification and anti-tumour activity of cyanidin-3-*O*-glucoside from Chinese bayberry fruit, Food Chem. **131** (2012) 1287–1294.

[6] A. Tarozzi, F. Morroni, A. Merlicco, C. Bolondi, G. Teti, M. Falconi, G. Cantelli-Forti, P. Hrelia, Neuroprotective effects of cyanidin 3-*O*-glucopyranoside on amyloid beta (25-35) oligomer-induced toxicity, Neurosci. Lett. **473** (2010) 72–76.

[7] A. Tarozzi, F. Morroni, S. Hrelia, C. Angeloni, A. Marchesi, G. Cantelli-Forti, P. Hrelia, Neuroprotective effects of anthocyanins and their *in vivo* metabolites in SH-SY5Y cells, Neurosci. Lett. **424** (2007) 36–40.

[8] S. Chaudhuri, A. Banerjee, K. Basu, B. Sengupta, P.K. Sengupta, Interaction of flavonoids with red blood cell membrane lipids and proteins: Antioxidant and antihemolytic effects, Int. J. Biol. Macromol. **41** (2007) 42–48.

[9] M.H. Grace, D.M. Ribnicky, P. Kuhn, A. Poulev, S. Logendra, G.G. Yousef, I. Raskin, M. A. Lila, Hypoglycemic activity of a novel anthocyanin-rich formulation from lowbush blueberry, Vaccinium angustifolium Aiton, Phytomedicine **16** (2009) 406–415.

[10] H.A. Hassan, A.F. Abdel-Aziz, Evaluation of free radical-scavenging and anti-oxidant properties of black berry against fluoride toxicity in rats, Food Chem. Toxicol. **48** (2010) 1999–2004.

[11] N. Yao, F. Lan, R.-R. He, H. Kurihara, Protective Effects of Bilberry (*Vaccinium myrtillus* L.) Extract against Endotoxin-Induced Uveitis in Mice, J. Agric. Food Chem. **58** (2010) 4731–4736.

[12] A. Bishayee, T. Mbimba, R.J. Thoppil, E. Háznagy-Radnai, P. Sipos, A.S. Darvesh, H.G. Folkesson, J. Hohmann, Anthocyanin-rich black currant (*Ribes nigrum* L.) extract affords chemoprevention against diethylnitrosamine-induced hepatocellular carcinogenesis in rats, J. Nutr. Biochem. **22** (2011) 1035–1046.

[13] F. Tremblay, J. Waterhouse, J. Nason, W. Kalt, Prophylactic neuroprotection by blueberry-enriched diet in a rat

model of light-induced retinopathy, J. Nutr. Biochem. **24** (2013) 647–655.

[14] T.H. Marczylo, D. Cooke, K. Brown, W.P. Steward, A.J. Gescher, Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice, Cancer Chemother. Pharmacol. **64** (2009) 1261––1268.

[15] J. Zawistowski, A. Kopec, D.D. Kitts, Effects of a black rice extract (Oryza sativa L. indica) on cholesterol levels and plasma lipid parameters in Wistar Kyoto rats, J. Funct. Foods **1** (2009) 50–56.

[16] A. Sarić, S. Sobocanec, T. Balog, B. Kusić, V. Sverko, V. Dragović-Uzelac, B. Levaj, Z. Cosić, Z. Macak Safranko, T. Marotti, Improved Antioxidant and Anti-inflammatory Potential in Mice Consuming Sour Cherry Juice (*Prunus Cerasus* cv. Maraska), Plant Foods Hum. Nutr. **64** (2009) 231–237.

[17] X. Yang, L. Yang, H. Zheng, Hypolipidemic and antioxidant effects of mulberry (Morus alba L.) fruit in hyperlipidaemia rats, Food Chem. Toxicol. **48** (2010) 2374––2379.

[18] M.F.-F. Chong, R. Macdonald, J. a Lovegrove, Fruit polyphenols and CVD risk: a review of human intervention studies, Br. J. Nutr. **104** Suppl (2010) S28–S39.

[19] P.-N. Chen, S.-C. Chu, H.-L. Chiou, C.-L. Chiang, S.-F. Yang, Y.-S. Hsieh, Cyanidin 3-Glucoside and Peonidin 3-Glucoside Inhibit Tumor Cell Growth and Induce Apoptosis *In Vitro* and Suppress Tumor Growth *In Vivo*, Nutr. Cancer **53** (2005) 232–243.

[20] M.N. Clifford, Anthocyanins – nature, occurrence and dietary burden, J. Sci. Food Agric. **80** (2000) 1063–1072.

[21] K. Torskangerpoll, Ø.M. Andersen, Colour stability of anthocyanins in aqueous solutions at various pH values, Food Chem. **89** (2005) 427–440.

[22] P. Bridle, C.F. Timberlake, Anthocyanins as natural food colours—selected aspects, Food Chem. **58** (1997) 103––109.

[23] G. Mazza, R. Brouillard, Color Stability and Structural Transformations of Cyanidin 3,5-Diglucoside and Four 3-Deoxyanthocyanins in Aqueous Solutions, J. Agric. Food Chem. **35** (1987) 422–426.

[24] F.J. Heredia, E.M. Francia-Aricha, J.C. Rivas-Gonzalo, I.M. Vicario, C. Santos-Buelga, Chromatic characterization of anthocyanins from red grapes - I. pH effect , Food Chem. **63** (1998) 491–498.

[25] C. Del Carpio Jiménez, C. Serrano Flores, J. He, Q. Tian, S.J. Schwartz, M.M. Giusti, Characterisation and preliminary bioactivity determination of Berberis boliviana Lechler fruit anthocyanins, Food Chem. **128** (2011) 717––724.

[26] G. Mazza, E. Miniati, Anthocyanins in Fruits, Vegetables, and Grains, in: CRC Press, Boca Raton, FL, 1993, pp. 1––28.

[27] G.H. Laleh, H. Frydoonfar, R. Heidary, R. Jameei, S. Zare, The Effect of Light, Temperature, pH and Species on Stability of Anthocyanin Pigments in Four Berberis Species, Pakistan J. Nutr. **5** (2006) 90–92.

[28] M.T. Bordignon-Luiz, C. Gauche, E.F. Gris, L.D. Falcão, Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems, LWT – Food Sci. Technol. **40** (2007) 594–599.

[29] A. Downham, P. Collins, Colouring our foods in the last and next millennium, Int. J. Food Sci. Technol. **35** (2000) 5–22.

[30] R. Brouillard, G.A. Iacobucci, J.G. Sweeny, Chemistry of Anthocyanin Pigments. 9. UV-Visible Spectrophotometric Determination of the Acidity Constants of Apigeninidin and Three Related 3-Deoxyflavylium Salts, J. Am. Chem. Soc. **104** (1982) 7585–7590.

[31] R. Drabent, B. Pliszka, G. Huszcza-Ciołkowska, B. Smyk, Ultraviolet Fluorescence of Cyanidin and Malvidin Glycosides in Aqueous Environment, Spectrosc. Lett. **40** (2007) 165–182.

[32] P. Figueiredo, J.C. Lima, H. Santos, M. Wigand, R. Brouillard, A.L. Maqanita, F. Pina, Photochromism of the Synthetic 4',7-Dihydroxyflavylium Chloride, J. Am. Chem. Soc. **116** (1994) 1249–1254.

[33] P. Figueiredo, M. Elhabiri, N. Saito, R. Brouillard, Anthocyanin Intramolecular Interactions. A New Mathematical Approach To Account for the Remarkable Colorant Properties of the Pigments Extracted from Matthiola incana, J. Am. Chem. Soc. **118** (1996) 4788–4793.

[34] F. George, P. Figueiredo, R. Brouillard, Malvin Z-chalcone: An unexpected new open cavity for the ferric cation, Phytochemistry **50** (1999) 1391–1394.

[35] P.F. da Silva, J.C. Lima, F.H. Quina, A.L. Maçanita, Excited-State Electron Transfer in Anthocyanins and Related Flavylium Salts, J. Phys. Chem., A **108** (2004) 10133–10140.

[36] H. Santos, D.L. Turner, J.C. Lima, P. Figueiredo, F.S. Pina, A.L. Maçanita, Elucidation of the Multiple Equilibria of Malvin in Aqueous Solution by One- and Two-Dimensional NMR, Phytochemistry **33** (1993) 1227–1232.

[37] R. Drabent, B. Pliszka, T. Olszewska, Fluorescence properties of plant anthocyanin pigments. I. Fluorescence of anthocyanins in Brassica oleracea L. extracts, J. Photochem. Photobiol., B **50** (1999) 53–58.

[38] P. Figueiredo, F. Pina, L. Vilas-Boas, A.L. Macanita, Fluorescence spectra and decays of malvidin 3,5-diglucoside in aqueous solutions, J. Photochem. Photobiol., A **52** (1990) 411–424.

[39] N.J. Cherepy, G.P. Smestad, M. Grätzel, J.Z. Zhang, Ultrafast Electron Injection: Implications for a Photoelectrochemical Cell Utilizing an Anthocyanin Dye-Sensitized TiO2 Nanocrystalline Electrode, J. Phys. Chem., B. **101** (1997) 9342–9351.

[40] G. Agati, P. Matteini, J. Oliveira, V. de Freitas, N. Mateus, Fluorescence Approach for Measuring Anthocyanins and Derived Pigments in Red Wine, J. Agric. Food Chem. **61** (2013) 10156–10162.

[41] G. Agati, C. D'Onofrio, E. Ducci, A. Cuzzola, D. Remorini, L. Tuccio, *et al.*, Potential of a Multiparametric Optical Sensor for Determining *in Situ* the Maturity Components of Red and White Vitis vinifera Wine Grapes, J. Agric. Food Chem. **61** (2013) 12211–12218.

[42] R. Brouillard, B. Delaporte, J.-E. Dubois, Chemistry of Anthocyanin Pigments. 3. Relaxation Amplitudes in

pH-Jump Experiments, J. Am. Chem. Soc. **100** (1978) 6202–6205.

[43] R. Brouillard, Flavonoids and flower colour, in: J.B. Harbone (Ed.), Flavonoids, Adv. Res. Since 1980, Chapman and Hall, London, 1988, p. 525.

[44] R. Brouillard, G. Mazza, Z. Saad, A.M. Albrecht-Gary, A. Cheminat, The Copigmentation Reaction of Anthocyanins: A Microprobe for the Structural Study of Aqueous Solutions, J. Am. Chem. Soc. **111** (1989) 2604–2610.

[45] M. Friedman, H.S. Jürgens, Effect of pH on the stability of plant phenolic compounds., J. Agric. Food Chem. **48** (2000) 2101–2110.

[46] R. Brouillard, Anthocyanins as Food Colors, Academic Press, New York, 1982.

[47] G. Mazza, R. Brouillard, The mechanism of co-pigmentation of anthocyanins in aqueous solutions, Phytochemistry **29** (1990) 1097–1102.

[48] P. Figueiredo, M. Elhabiri, K. Toki, N. Saito, O. Dangles, R. Brouillard, New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for colour loss?, Phytochemistry **41** (1996) 301–308.

IZVOD

PROUČAVANJE FLUORESCENTNIH SVOJSTAVA CIJANIDINA I CIJANIDIN 3-*O*-*β*-GLUKOPIRANOZIDA

Violeta P. Rakić[1], Ajda M. Ota[2], Mihaela A. Skrt[2], Milena N. Miljković[3], Danijela A. Kostić[3], Dušan T. Sokolović[4], Nataša E. Poklar Ulrih[2,5]

[1]*Visoka poljoprivredno–prehrambena škola strukovnih studija, Prokuplje, Srbija*
[2]*Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[3]*Departman za hemiju, Prirodno–matematički fakultet, Univerzitet u Nišu, Niš, Srbija*
[4]*Medicinski fakultet, Univerzitet u Nišu, Niš, Srbija*
[5]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CipKeBiP), Ljubljana, Slovenia*

(Naučni rad)

U ovom radu ispitivana su apsorpciona i fluorescentna svojstva aglikona bez šećera, cijanidina i uticaj njegove 3-glukozidne supstitucije (do oblika Cy3Glc) na spektralne parametre. Učinjen je pokušaj da se utvrdi korelacija između apsorpcionih i fluorescentnih emisionih spektara. Ekscitacija molekula cijanidina i Cy3Glc vršena je talasnim dužinama iz UV oblasti spektra (210–350 nm, na svakih 10 nm). Poređenje apsorpcionog spektra cijanidina pri pH 5,5 sa apsorpcionim spektrom flavilijum katjona (pH 0,4) pokazuje da u vodenom rastvoru, pri pH 5,5, uglavnom postoji bezbojna forma cijanidina. Emisioni spektri cijanidina proučavani su pri ekcitaciji molekula talasnim dužinama iz UV oblasti spektra, koje su odgovarale talasnim dužinama apsorpcionih maksimuma. U skladu sa UV–Vis apsorpcionim spektrima, najefikasnija ekscitacija molekula cijanidina izmerena je na 220 i 230 nm, gde je molarna apsorptivnost jedinjenja najveća. Druga efikasna ekscitacija molekula cijanidina bila je na 270 i 280 nm. Utvrđeno je da cijanidin ima fuorescentni emisioni spektar sa dve trake, prvu u UV oblasti sa maksimumom na 310 nm i drugu u vidljivoj oblasti sa maksimumom na 615 nm. Poređenje apsorpcionog spektara Cy3Glc pri pH 5,5 sa apsorpcionim spektrom flavilijum katjona (pri pH 0,4) ukazuje na toda Cy3Glc uglavnom postoji u bezbojnom obliku pri ovoj pH vrednosti. U skladu sa UV–Vis apsorpcionim spektrima, najefikasnija ekscitacija molekula Cy3Glc izmerena je na 220 i 230 nm, gde je molarna apsorptivnost jedinjenja najveća. Druga efikasna ekscitacija molekula Cy3Glc bila je pri 300 i 310 nm. Utvrđeno je da Cy3Glc ima fluorescentni emisioni spektar sa maksimumom na 380 nm, ali ne pokazuje fluorescentnu emisiju u vidljivoj oblasti. Ako se uporede fluorescentni emisioni spektri cijanidina i Cy3Glc može se videti da je intenzitet fluorescentne emisije cijanidina značajno veći od intenziteta fluorescentne emisije Cy3Glc. Ovi rezultati ukazuju na uticaj 3-glukozidne supstitucije na C-3 atomu aglikona (do oblika Cy3Glc) na značajano smanjenje intenziteta fluorescentne emisije i gašenje fluorescencije u oblasti talasnih dužina vidljivog dela spektra.

Ključne reči: Antocijanini • Antocijanidini • Cijanidin • Cijanidin 3-glukopiranozid • Fluorescentni emisioni spektar • UV–Vis apsorpcioni spektar

Procena uticaja rudničkih drenažnih i komunalnih otpadnih voda na kvalitet površinskih voda u Boru i okolini

Vojka R. Gardić[1], Jelena V. Petrović[1], Lidija V. Đurđevac-Ignjatovic[1], Srđan R. Kolaković[2], Svetlana R. Vujović[2]

[1]Institut za rudarstvo i metalurgiju Bor, Bor, Srbija
[2]Fakultet tehničkih nauka, Departman za građevinarstvo, Univerzitet u Novom Sadu, Novi Sad, Srbija

Izvod

Rudarstvo i proizvodnja bakra u Boru, tokom proteklih sto godina, imalo je ogroman uticaj na životnu okolinu. Otpadne vode koje se generišu u pogonima RTB Bor zagađuju Borsku i Kriveljsku reku koje predstavljaju otvoreni kolektor za otpadnu vodu. Posle uliva Borske reke u Kriveljsku reku nastaje Bela reka koja se dalje uliva u reku Timok, odnosno reku Dunav. Za period istraživanja od 17. oktobra 2012. god. do 17. januara 2013. sprovedena je analiza rudničkih, drenažnih i komunalnih otpadnih voda. Za potrebe istraživanja i procenu negativnog uticaja otpadnih voda na površinske vode na terenu je sprovedeno merenje protoka, temperature, pH vrednosti, oksido/redukcionog potencijala i koncentracije rastvornog kiseonika u uzorcima vode. Takođe, određen je i sadržaja Cu, Fe^{2+}, ukupnog Fe (T-Fe), As, Mn, Si, Ni i Al.

Ključne reči: rudarstvo, otpadne vode, površinske vode, hemijske karakteristike.

STRUČNI RAD

UDK 622.34(497.11Bor)628.3:504.5

Hem. Ind. **69** (2) 165–174 (2015)

doi: 10.2298/HEMIND140128031G

Bor je smešten u dolini Borske reke na nadmorskoj visini od 360 m. Bor i okolina su poznati po najvećim nalazištima bakra u Srbiji i svrstana su među najveća u Evropi [1]. Borski rudnik sa otvorenim kopom se nalazi na severnoj granici Bora. Dubok je oko 300 m, a širok preko 1 km na najširem delu. Rudarske aktivnosti se ne upražnjavaju i kop se sada koristi kao skladište jalovine koja se doprema od rudnika Veliki Krivelj zajedno sa šljakom iz topionice. Iskop otvorenog kopa rudnika Veliki Krivelj započeo je 1979, a proizvodnja je u njemu počela 1982. godine. Od tada je iskopano oko 150 miliona tona rude. Proizvodnja se nastavlja stopom od oko 4,8 miliona tona godišnje [2]. Na području Bora, u zoni delovanja Rudarsko topioničarskog basena (RTB), degradirano je preko 29.000 ha zemljišta pod šumama i oranicama. Površina degradiranog zemljišta, od ukupnog poljoprivrednog zemljišta u borskoj opštini, iznosi preko 60% [3]. Površinske i podzemne vode u blizini bazena sa jalovinom se mogu kontaminirati teškim metalima kao što su olovo, cink, bakar i arsen, koji su već nađeni u zemljištu i biljkama u borskom regionu [4]. Otpadne vode koje se generišu u pogonima RTB Bor zagađuju Borsku i Kriveljsku reku, koja se dalje uliva u reku Timok, odnosno reku Dunav. Ova zagađenja su najčešće predstavljena niskom pH vrednošću, povećanim sadržajem jona teških metala, suspendovanim česticama i finim česticama flotacijske jalovine koja je deponovana u dolinama navedenih reka na površini od preko 2000 hektara [5]. Tokom višedecenijske eksploatacije rude sa površinskog kopa Bor na različitim lokalitetima („Visoki planir“ – nazvan i Oštreljski planir, „Severni planir“, rudnička deponija rudnog tela „H“ (RTH)) odlagale su se raskrivka i jalovina. Najveća količina jalovine, oko 150 miliona tona, odložena je na Visokim planirima. Procenjen je sadržaj bakra od 0,15%, od čega 20% može da se predstavi kao bakar u obliku oksidnih minerala, dok su od sulfidnih minerala prisutni halkozin, kovelin i neznatan sadržaj halkopirita. Cilj ovog rada da se na osnovu hemijskih karakteristika rudničkih drenažnih i komunalnih otpadnih voda u Boru i okolini, proceni uticaj na na kvalitet površinskih voda, čiji je kvalitet takođe analiziran. U radu su prikazani rezultati dobijeni za period istraživanja od 17. oktobra 2012. do 17. januara 2013. godine.

UTICAJ RUDARSKIH AKTIVNOSTI NA ŽIVOTNU SREDINU

Prema rezultatima istraživanja iz 1989. godine, procenjeno je da je oko 19,300 km potoka i reka, kao i 72,000 ha jezera i rezervoara širom sveta bilo ozbiljno ugroženo otpadnim vodama poreklom iz rudnika [7]. Kisele drenažne vode, proceđivanje iz jalovišta ili direktno odlaganje jalovine u vodotokove može uzrokovati zagađenje površinskih i podzemnih voda [8]. Uticaj kiselih drenažnih voda na vodotokove može ozbiljno narušite kvalitet vode. Ozbiljnost i stepen degradacije vodotoka u zavisnosti je od različitog broja faktora kao što su: učestalost, zapremina i hemijske karakteristike rudničkih drenažnih voda. Takođe, značajan

---

Prepiska: S. Vujović, Departman za građevinarstvo, Fakultet tehničkih nauka, Univerzitet u Novom Sadu, Novi Sad, Trg Dositeja Obradovića 6, 21 000 Novi Sad, Srbija.
E-pošta: vujovics@uns.ac.rs

Rad primljen: 28. januar, 2013
Rad prihvaćen: 7. april, 2014

faktor je i puferski kapacitet recipijenta kao i udaljenost recipijenta od izvora zagađenja [9]. Uticaj zagađenja kiselih rudničkih voda na životnu sredinu je kompleksan. Osnovni efekti su: toksičnost metala; proces taloženja, kiselost i salinizacija [10]. Kisele rudničke vode su osnovna pokretačka sila odgovorna za zagađenje teškim metalima. Kisele rudničke vode utiču na oslobađanje metala iz mineralnih ruda u životnu sredinu čineći ih dostupnim za akvatičnie organizame. Teški metali se iz akvatičnog ekosistema ne mogu ukloniti procesima samoprečišćavanja, već se akumuliraju u sedimentu gde mogu ući u lanac ishrane biomagnifikacijom. Stoga, sediment predstavlja značajan izvor teških metala [11]. Vrednost pH je značajan parametar kvaliteta voda. Za većinu akvatičnih organizama optimalna pH vrednost se kreće od 7,2–8,7. Kako visoke vrednosti pH utiču na rastvorljivost teških metala, a samim tim i na njihovu toksičnost, postoji opasnost od oslobađanja metalnih jona (npr. aluminijuma) iz kompleksa sa drugim katjonima. pH vrednost vode određuje rastvorljivost i biološku raspoloživost hemijskih konstituenata azota. Ukoliko su vrednosti pH niske u vodi su prisutni amonijum jon (NH_4^+) i hidronijum jon (H_3O^+). Amonijum jon nije toksičan za žive organizme. Međutim, opasnost za živi svet postoji u slučaju većih vrednosti pH (iznad 9) kada je dominantan NH_3 kao toksična komponenta [12,13].

Oblast istraživanja

Istraživanjem su obuhvaćeni sledeći izvori zagađenja: otpadne rudničke drenažne vode koje potiču iz aktivnih rudnika (borska jama, polje 1 jalovišta Veliki Krivelj, flotacijsko jalovište RTH u Boru, metalurške vode), kao i drenažne vode iz flotacijskih jalovišta koja nisu više u funkciji (polje 2 flotacijskog jalovišta Veliki Krivelj, drenažne vode iz starog Borskog flotacijskog jalovišta), stara neaktivna odlagališta kopovske raskrivke (Saraka odlagalište, Visoki planiri – jalovina iz starog Borskog kopa, odlagalište kopovske raskrivke iz kopa RTH) i gradske – komunalne otpadne vode, koje se bez prečišćavanja ispuštaju direktno u vodotok Borske reke. Navedene otpadne vode direktno zagađuju Borsku i Krivaljsku reku, koje nakon spajanja kod mosta nizvodno od sela Slatina formiraju Belu reku. Uzorkovanje otpadnih i rečnih voda sprovedeno je na sledećim lokacijama: rudničke drenažne vode iz borskog rudnika i jalovine, otpadne vode iz topionice i gradske-komunalne otpadne vode koje su uzorkovane u neposrednoj blizini borskog rudnika. Takođe, za potrebe istraživanja uzeti su uzorci rečnih voda. Uzorkovanje i ispitivanje je sprovedeno na ukupno 17 lokacija. Lokacije uzorkovanja prikazane na slici 1 i date su sledećim oznakama:

P-1 Kriveljska reka sa drenažnom vodom sa površinskog kopa Veliki Krivelj;

P-2 Saraka potok;

P-3 Otpadne vode iz Jame Bor;

P-4 Kriveljska reka pre kriveljskog jalovišta;

P-5 Kriveljska reka nakon kriveljskog jalovišta;

P-6-1 Metalurške otpadne vode;

P-6-2 Komunalne otpadne vode;

P-6-3 Drenažne vode sa raskrivke (jalovine) u području RTH jalovišta;

P-8 Cerova reka južno od površinskog kopa Cerovo;

P-9 Borska reka;

P-10 Kriveljska reka;

P-11 Jezero Robule;

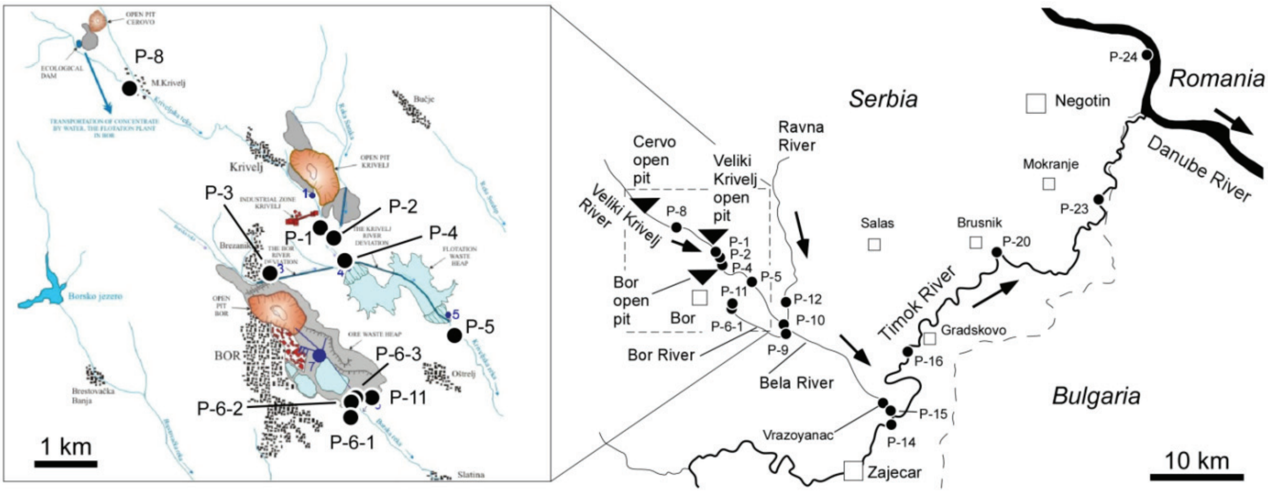
P-12 Ravna reka;

P-13 Bela reka posle spajanja Borske, Kriveljske i Ravne reke;

P-14 Reka Timok pre uliva Bele reke;

P-15 Bela reka pre uliva u reku Timok;

P-16 Reka Timok posle uliva Bele reke, most na Timoku, lokacija Čokonjar.



Slika 1. Lokacije uzorkovanja voda u regionu Bora i Zaječara.
Figure 1. Map of the sampling locations in the area of the cities of Bor and Zaječar.

EKSPERIMENTALNI DEO

Aanalitičke procedure uzoraka vode

Određivanje protoka, temperature, pH vrednosti, oksido/redukcionog potencijala (ORP) i koncentracije rastvornog kiseonika (DO) u uzorcima vode, za potrebe ovog istraživanja sprovedeno je na terenu. Određivanje sadržaja Cu, Fe^{2+}, ukupnog Fe (T-Fe), Mn, Si, Ni i Al u uzorcima sprovedeno je primenom kolorimetra (HACH Company, DR-890) u skladu sa Direktivom EU (Council Directive 79/869/EEC) [14]. Sadržaj Fe^{3+} je dobijen proračunom iz razlike koncentracije ukupnog sadržaja Fe i koncentracije Fe^{2+}. Sadržaj ukupnog arsena meren je na terenu upotrebom seta za određivanje arsena (LaMotte Company – Total As Kit).

REZULTATI I DISKUSIJA

Karakteristike rudničkih drenažnih, komunalnih otpadnih i rečnih voda u okolini borskog rudnika

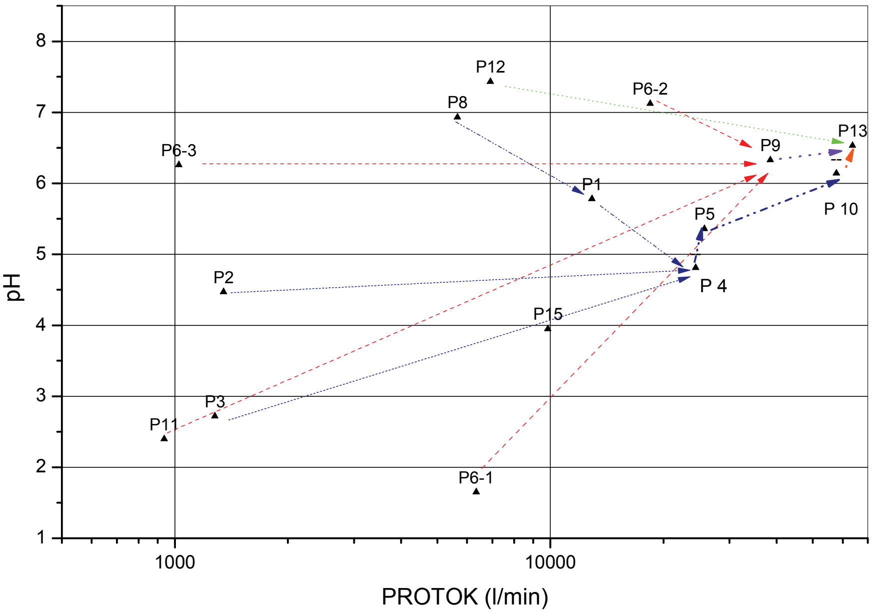
Za potrebe istraživanja sprovedenog na terenu vršeno je: određivanje protoka, temperature, pH vrednosti, oksido/redukcionog potencijala (ORP) i koncentracije rastvornog kiseonika (DO). Dijagram zavisnosti pH vrednosti od protoka za drenažne rudničke vode, otpadne vode i rečne vode u borskoj oblasti prikazan je na slici 2.

Vode iz jezera Robule i drenažne jamske vode (P-3), vode na ulazu u kolektor kriveljskog jalovišta (P-4) i metalurške otpadne vode (P-6-1) su kiselog karaktera, sa pH vrednostima nižim od zakonom propisanih vrednosti, prema Pravilniku o opasnim materijama u vodama (Sl. glasnik SRS, br. 31/82) [15]. Vode uzorkovane na tačkama P-11, P-3, P-4 i P-6-1 imaju pH vrednosti niže od zakonom propisanih u svakom izveštajnom periodu.

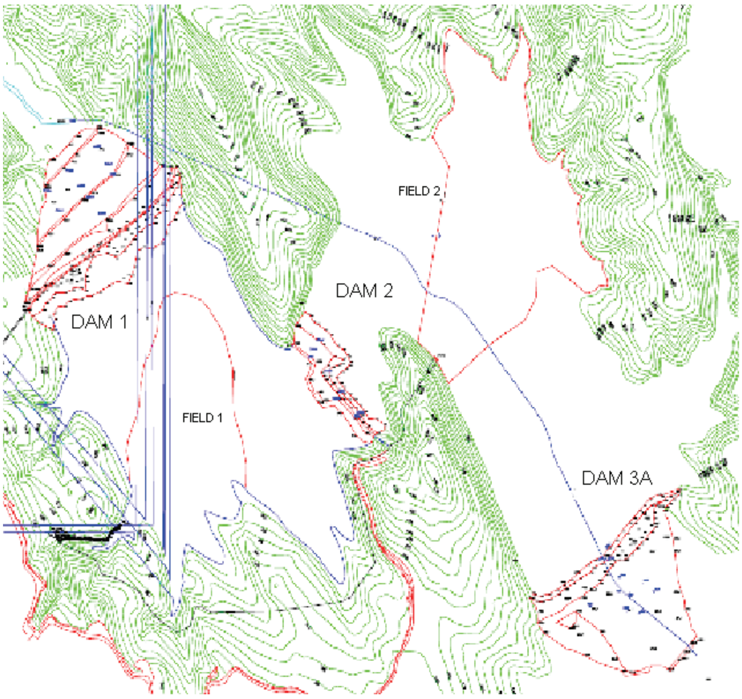
Vode na ulazu u kriveljski kolektor ispod brane 1A flotacijskog jalovišta (Slika 3) Veliki Krivelj (P-1) nastaju spajanjem Saraka potoka (P-2), Kriveljske Reke (P-10), jamskih otpadnih voda (P-3) i manjim udelom drenažnih voda brane 1A, te se može zaključiti da jamske otpadne vode imaju najveći uticaj na kiselost vode na mernoj tački P-4, nakon čega slede vode Saraka potoka (P-2). Na izlazu iz kriveljskog kolektora (P-5) dolazi do manjeg povećanja protoka, kao rezultat uliva drenažne vode brane 3A jalovišta Veliki Krivelj, dok pH vrednost ostaje gotovo nepromenjena. Drenažne vode brane 3A su najčešće neutralnog ili blago kiselog karaktera (imaju pH vrednost oko 7), ali zbog malog protoka u odnosu na protok Kriveljske reke, njihov uticaj na povećanje pH vrednosti u Kriveljskoj reci je zanemarljiv.

Metalurške otpadne vode su uz očekivanu nisku pH vrednost, imale i visoki sadržaj bakra i veliki protok. Za period ispitivanja, vode iz Saraka potoka (P-2) i otpadne rudničke vode iz jame Bor (P-3) pokazale su kiseli karakter, gde je izmerena vrednost pH od 4,47, odnosno 2,72. Za period istraživanja metalurške otpadne vode imale su najznačajniji uticaj na životnu sredinu i na rečni sistem Borska reka i Bela reka. Vrednost pH od 1,65 i koncentracija bakra od 156 mg/l potvrđuju navedene činjenice i predstavljaju maksimalno izmerene vrednosti za ceo period ispitivanja ovih voda.

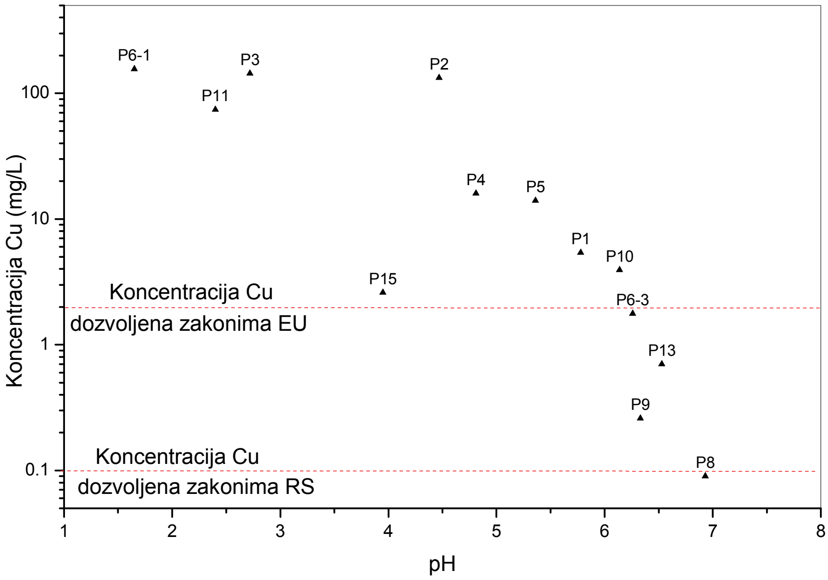
Dijagram zavisnosti pH vrednosti i sadržaja bakra u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama borske oblasti prikazan je na slici 4. Sadržaj bakra u drenažnim vodama iz jame i otpadnim vodama u borskoj oblasti kreće se od BDL (eng. *below detection*



Slika 2. Dijagram zavisnosti pH vrednosti od protoka za drenažne rudničke, otpadne i rečne vode u borskoj oblasti.
Figure 2. Diagram of the pH versus flow of mine drainage water, wastewater and river water in the area of the city of Bor.



Slika 3. Situaciona karta starog flotacijskog jalovišta.
Figure 3. Layout map of the old flotation tailings.



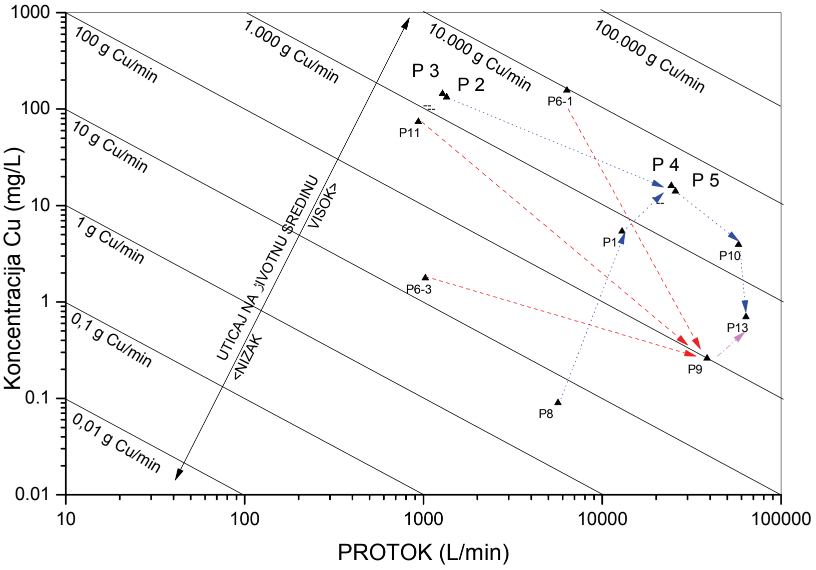
Slika 4. Dijagram zavisnosti pH vrednosti i sadržaja bakra u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama borske oblasti.
Figure 4. Diagram of the pH versus copper concentration of mine drainage water, wastewater and river water in the area of the city of Bor.

limit) – ispod granice detekcije kolorimetra (P-6-2) do 156 mg/l (P-6-1).

Koncentracija bakra u rečnoj vodi u borskoj oblasti, za izveštajni period kretala se od BDL (P-12, P-14 i P-16) do 16 mg/l (P-4).

Dijagram zavisnosti koncentracije bakra i protoka u rudničkim drenažnim vodama, otpadnim vodama i površinskim rečnim vodama borske oblasti prikazan je na slici 5.

Vode sa velikim protokom i visokom koncentracijom bakra su otpadne metalurške vode (P-6-1) i jamske otpadne vode (P-3). Visoka koncentracija bakra je izmerena i u vodama Saraka potoka, tačka P-2 (133 mg/l), ali je protok ovih voda mali, čime se direktno umanjuje i negativan uticaj ovih voda na okolinu. Takođe, kao što

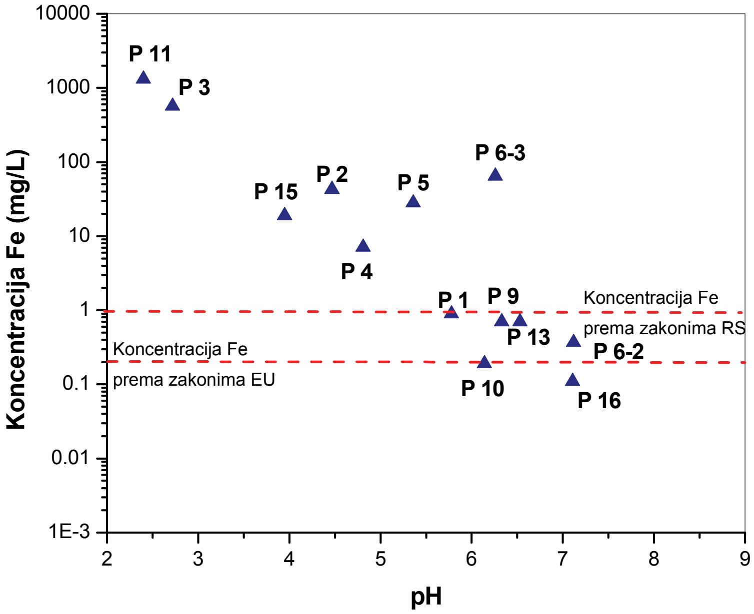


Slika 5. Dijagram zavisnosti koncentracije bakra i protoka u rudničkim drenažnim vodama, otpadnim vodama i površinskim rečnim vodama borske oblasti.
Figure 5. Diagram of the copper concentration versus flow of mine drainage water, wastewater and river water in the area of the city of Bor.

je prikazano na slici 4, jasno se uočava da samo vode uzorkovane u tačkama P-8, P-6-2, P-12, P-14 i P-16 sadrže bakar u koncentracijama ispod maksimalno dozvoljenih prema Pravilniku o opasnim materijama u vodama (Sl. glasnik SRS, br. 31/82) [15]. Ukoliko se sadržaj bakra u vodama uporedi sa EU zakonodavstvom (Direktiva 2006/11/EC; Direktiva 75/440/EEC) [16,17], pored navedenih, dozvoljene koncentracije bakra imaju i vode uzorkovane u tačkama P-6-3, P-9 i P-13.

Dijagram zavisnosti koncentracije ukupnog sadržaja gvožđa i pH vrednosti u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama u borskoj oblasti prikazan je na slici 6.

Sadržaj ukupnog gvožđa u zavisnosti od pH vrednosti u drenažnim rudničkim vodama i ostalim otpadnim vodama koje su u direktnoj vezi sa rudarskim aktivnostima, kreću se u opsegu od BDL do 1320 mg/l. Otpadne vode sa najvećom koncentracijom gvožđa su



Slika 6. Dijagram zavisnosti koncentracije ukupnog gvožđa i pH vrednosti u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama u borskoj oblasti.
Figure 6. Diagram of of the total iron concentration versus pH of mine drainage water, wastewater and river water in the area of the city of Bor.

sledeće: vode iz jezera Robule sa 1320 ppm, rudničke otpadne vode iz jame Bor sa 570 ppm, i metalurške vode sa 300 ppm. Rudničke otpadne vode iz jame Bor i metalurške vode izdvavaju se po povećanim koncentracijama jona gvožđa, slično kao kada je u pitanju koncentracija bakra. Vode iz jezera Robule, iako sadrže daleko više ukupnog gvožđa nego prethodne dve navedene vode, zbog veoma malog protoka ima manji štetni uticaj na životnu sredinu od navedenih otpadnih voda, koje nastaju kao nus-proizvod tekuće proizvodnje bakra u RTB Bor.

Dijagram zavisnosti koncentracije ukupnog gvožđa i protoka u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama u borskoj oblasti prikazan je na slici 7.

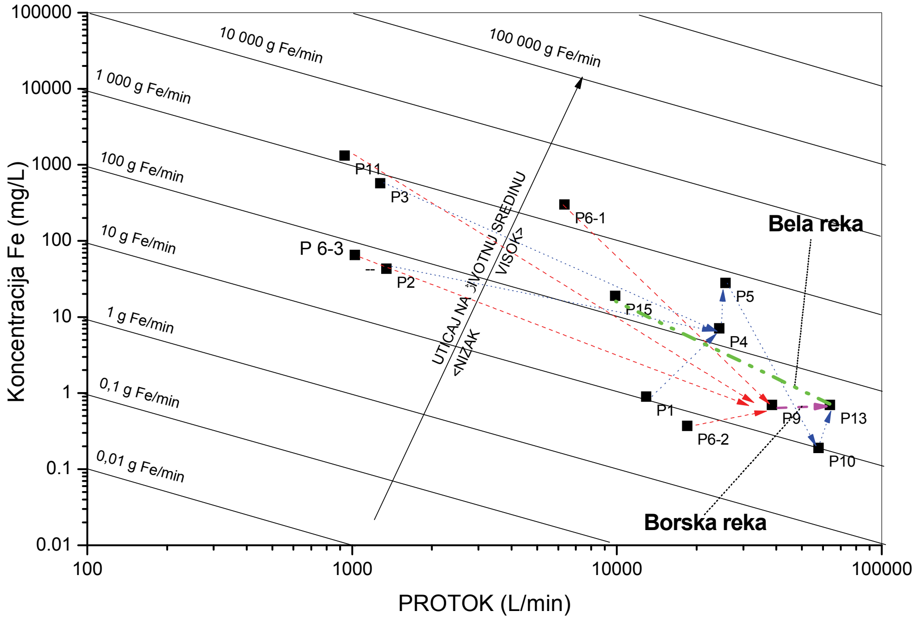
U površinskim vodama reka, sadržaj ukupnog gvožđa u borskoj oblasti kreće se u opsegu od BDL do 28 mg/L (tačka P-5). Vode sa velikim protokom i velikim sadržajem gvožđa su otpadne metalurške vode (P-6-1). Drenažne rudničke vode iz Robule jezera (P-11) imaju mali protok, ali je sadržaj ukupnog gvožđa iznad maksimalno dozvoljene koncentracije, prema Pravilniku o opasnim materijama u vodama (Sl. glasnik SRS, br. 31/82) [15]. Vode uzorkovane u tačkama P-10 i P-16 imaju dozvoljene koncentracije gvožđa, prema zakonodavstvu EU (Direktiva 2006/11/EC; Direktiva 75/440/ /EEC) [16,17]. U tačkama P-1, P-6-2, P-9 i P-13, sadržaj gvožđa je u skladu sa Pravilnikom o opasnim materijama u vodama (Sl. glasnik SRS, br. 31/82) [14].

Određivanje sadržaja ukupnog arsena izvršeno je na svih 16 uzoraka vode. Dijagram zavisnosti koncentracije As od pH vrednosti u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama u borskoj oblasti prikazan je na slici 8.

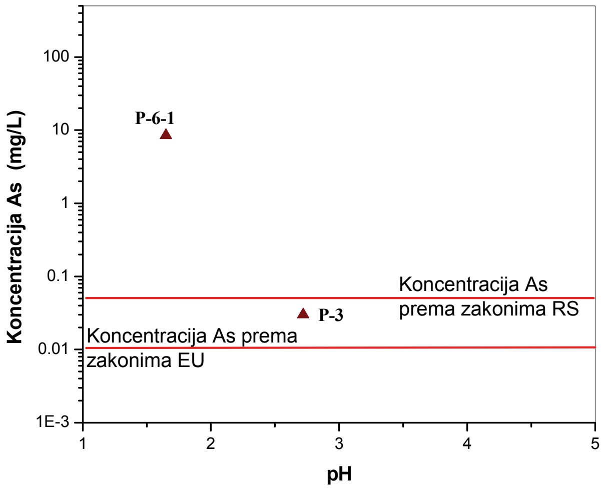
Povišen sadržaj As je uočen u metalurškim otpadnim vodama (P-6-1) i otpadnim vodama jame Bor (P-3). Sadržaj arsena u otpadnim metalurškim vodama je 8,5 ppm, a u jamskim otpadnim vodama je 0,030 ppm. Izmerene koncentracije arsena u tački P-6-1 ima tendenciju rasta, u poređenju sa vrednostima izmerenim u prethodnim izveštajnim periodima. Arsen nije detektovan u vodama koje su uzorkovane niže od tačke P-6-1, što je sa jedne strane direktan uticaj faktora razblaženja od oko 6 puta, posmatrajući protoke u tačkama P-6-1 i P-9, dok sa druge strane predstavlja i uticaj sedimentacije arsena na dnu rečnog korita. Ovaj uticaj je potvrđen analizom sedimenata i zemljišta u borskoj oblasti u prethodnom periodu istraživanja [6]. Prema Pravilniku o opasnim materijama u vodama (Sl. glasnik SRS, br. 31/82) [14] izmerena koncentracija arsena od 8,5 ppm je 170 puta veća od maksimalno dozvoljene koncentracije arsena u vodama. Takođe, izmerena koncentracija arsena je 850 puta veća od maksimalno dozvoljene koncentracije arsena u vodama prema zakonodavstvu EU (Direktiva 2006/11/EC; Direktiva 75/440/EEC) [16,17].

Karakterizacija rečnih voda koje sadrže drenažne rudničke vode i otpadne vode iz oblasti borskog rudnika

Vrednosti za pH u rečnim vodama koje nisu pod uticajem rudničkih drenažnih voda i otpadnih voda iz borske oblasti je oko 7–8. U tački uzorkovanja P-8, koja

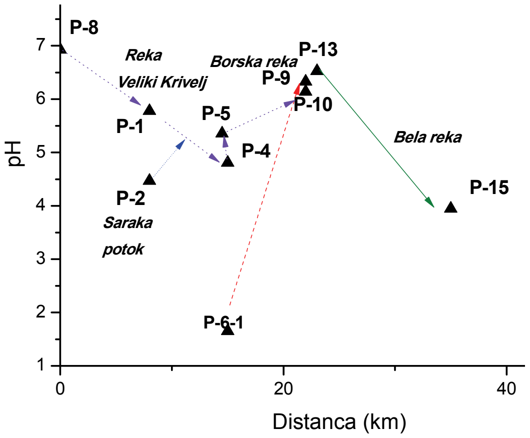


Slika 7. Dijagram zavisnosti koncentracije ukupnog gvožđa i protoka u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama u borskoj oblasti.
Figure 7. Diagram of the total iron concentration versus flow of mine drainage water, wastewater and river water in the area of the city of Bor.



Slika 8. Dijagram zavisnosti koncentracije As od pH vrednosti u rudničkim drenažnim vodama, otpadnim vodama i rečnim vodama u borskoj oblasti.
Figure 8. Diagram od the Arsenic concentration versus pH of mine drainage water, wastewater and river water in the area of the city of Bor.

je prelazna merna tačka između površinskog kopa Cerovo i površinskog kopa Veliki Krivelj na Kriveljskoj reci, izmerena je vrednost pH od 6,93. Takođe, tačka uzorkovanja P-12, nije pod uticajem rudničkih drenažnih voda i otpadnih voda iz borske oblasti u kojoj je određena vrednost pH od 7,43 – Ravna reka. Voda iz Borske reke ima slabo kiseli karakter i pH vrednost od 6,33 (P-9). Otpadne metalurške vode imaju jak kiseli karakter, sa najnižom do sada izmerenom pH vrednošću od 1,65 i protokom od 6345,70 l/min i veliki uticaj na kvalitet vode u Borskoj reci. Voda iz Bele reke, na mestu uzorkovanja P-13 (gornji tok) ima pH vrednost 6,53, ali u mernoj tački P-15 (donji tok) je kiselija i ima vrednost pH od 3,95, što je uticaj dejstva nagomilane flotacijske jalovine u dolinama ovih reka. Kriveljska reka nakon spajanja sa Saraka potokom i drenažnim rudničkim vodama postaje kiselija. Vrednost pH se neznatno povećava na izlazu iz kolektora ispod jalovišta usled spajanja sa drenažnim vodama brane 3A flotacijskog jalovišta. Ovako kisela voda u Kriveljskoj reci veoma štetno utiče na već oštećeni kolektor ispod flotacijskog jalovišta Veliki Krivelj, dodatno ga razarajući, kako betonsku oplatu tako i samu armaturu, koju kisela voda rastvara, pa je na nekim mestima veoma oštećena ili je skoro i nema. Usled spajanja sa rečicama koje se ulivaju u nju, Kriveljska reka na mestu spajanja sa Borskom rekom ima pH vrednost od 6,14. Niska pH vrednost negativno utiče kako na okolno zemljište tako i na poljoprivredne kulture. Stoga, ova voda nije pogodna za zalivanje poljoprivrednih kultura u dolinama kroz koje prolazi. Dijagram promene pH vrednosti u zavisnosti od distance od borskog rudnika prikazan je na slici 9.



Slika 9. Dijagram promene pH vrednosti u zavisnosti od distance od borskog rudnika.
Figure 9. Diagram of pH values depending on the distance from the Bor mine.

Dijagram promene u koncentraciji bakra u rečnim vodama u borskoj oblasti u zavisnosti od distance od borskog rudnika do Timoka prikazan je na slici 10.

Sadržaj bakra u rečnoj vodi u tački uzorkovanja P-4, P-5 i P-10, duž Kriveljske reke pokazuje varijacije u opsegu od 3,93 do 16 mg/L. Sadržaj bakra u rečnoj vodi na mestu uzorkovanja P-9, Borska reka bio je 0,26 mg/l, što je manje nego u prošlim izveštajnim periodima, dok je protok reke imao tendenciju povećanja. Sadržaj bakra u rečnoj vodi na mestu uzorkovanja Bela reka (P-13) je 0,7 mg/l (uzvodno: nakon spajanja Kriveljske i Borske reke), dok je na tački uzorkovanja P-15 bila 2,6 mg/l (nizvodno: pre ulivanja Bele reke u Timok). Na slici 11 prikazana je promena koncentracije bakra u zavisnosti od pH vrednosti.

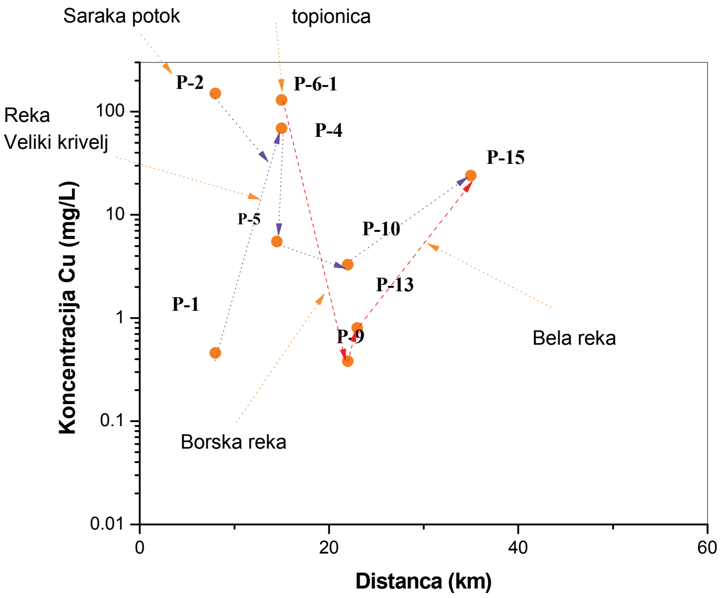
Kada su u pitanju drugi štetni metali, vode najzagađenije jonima nikla su metalurške vode sa 24,4 ppm i rudničkim drenažnim vodama iz borskog rudnika-Jama Bor sa 0,80 ppm.

Povećan sadržaj mangana se nalazi u vodama Saraka potoka sa 24,5 ppm, rudničkim otpadnim vodama iz borskog podzemnog rudnika – jama Bor sa 15,0 ppm, metalurškim vodama sa 14,1 ppm i jezera Robule sa 4,74 ppm [6].

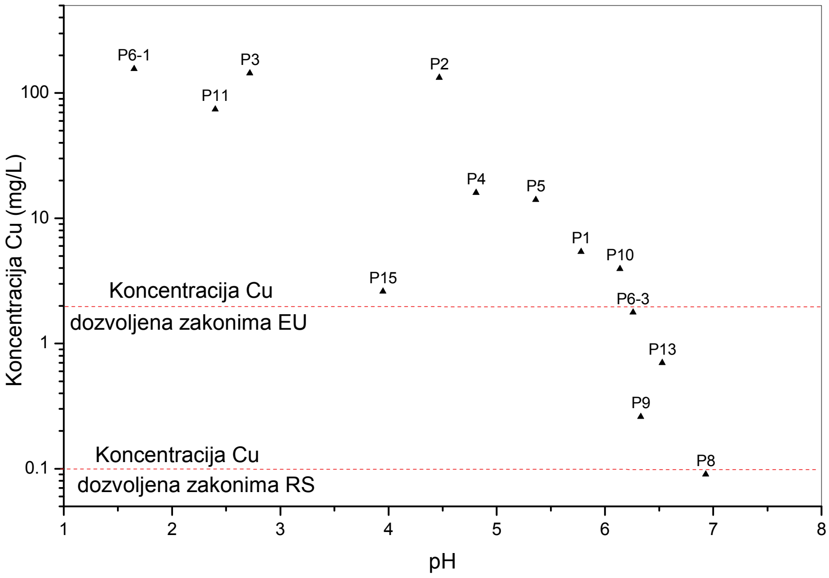
ZAKLJUČAK

Negativan uticaj rudarske aktivnosti na životnu sredinu je evidentan. Drenažne vode aktivnih rudnika, drenažne vode flotacijskih jalovišta koja više nisu u funkciji, stara neaktivna odlagališta kopovske raskrivke i neprečišćene otpadne vode direktno zagađuju Borsku i Krivaljsku reku, koje nakon spajanja kod mosta nizvodno od sela Slatina formiraju Belu reku.

Kriveljska reka nakon spajanja sa Saraka potokom i drenažnim rudničkim vodama postaje kiselija. Vrednost pH se neznatno povećava na izlazu iz kolektora ispod



Slika 10. Dijagram promene u koncentraciji bakra u rečnim vodama u borskoj oblasti u zavisnosti od distance od borskog rudnika do Timoka.
Figure 10. Diagram of Copper concentration depending on the distance of Bor mine from the Timok river.



Slika 11. Dijagram zavisnosti koncentracije bakra od pH vrednosti.
Figure 11. Diagram of Copper concentrations versus pH values.

jalovišta usled spajanja sa drenažnim vodama brane 3A flotacijskog jalovišta. Ovako kisela voda u Kriveljskoj reci veoma štetno utiče na već oštećeni kolektor ispod flotacijskog jalovišta Veliki Krivelj, dodatno ga razarajući, kako betonsku oplatu tako i samu armaturu, koju kisela voda rastvara, pa je na nekim mestima veoma oštećena ili je skoro i nema. Niska pH vrednost negativno utiče kako na okolno zemljište tako i na poljoprivredne kulture.

Dve najštetnije i najkiselije vode su vode koje aktivni rudnik (jama Bor) i TIR (metalurške vode) ispuštaju u Borsku i Kriveljsku reku bez prečišćavanja, što je u suprotnosti sa svim važećim zakonskim regulativama u našoj zemlji. Sprečavanjem njihovog daljeg ispuštanja i prečišćavanjem ovih jako kiselih i zagađenih voda znatno bi se poboljšao kvalitet voda u samim rekama, za veoma kratko vreme.

Sadržaj bakra u otpadnim vodama i rekama je u direktnoj vezi sa kiselim karakterom samih otpadnih voda. Najzagađeniji tokovi otpadnih voda bakrom su: metalurške vode sa 156 ppm, rudničke otpadne vode iz jamskog rudnika sa 144 ppm, Saraka potok sa 133 ppm i vode jezera Robule sa 74 ppm. Kao i kada je u pitanju pH vrednost otpadnih voda i u ovom slučaju se po zagađenosti izdvajaju otpadne vode iz aktivnih preduzeća i rudnika RTB Bor.

Sadržaj ukupnog gvožđa u drenažnm rudničkim vodama i ostalim otpadnim vodama koje su u direktnoj vezi sa rudarskim aktivnostima, kreću se u opsegu od BDL (P-8, P-12) do 1320 ppm za za jezero Robule (P-11).

Određivanje sadržaja ukupnog arsena izvršeno je na svih 16 uzoraka voda, a povišeni sadržaj je uočen u metalurškim otpadnim vodama (P-6-1) i rudničkim otpadnim vodama iz borskog rudnika – jama Bor (P-3). Dobijene vrednosti za As su najveće od kad se krenulo sa ispitivanjem kvaliteta otpadnih voda.

Zahvalnica

Ovaj rad je urađen okviru projekta „Management of mining waste-tailing dump in the Bor region“ podržanog od strane Japanskog društva za promociju nauke, Japanske internacionalne agencije za saradnju i Ministarstva za zaštitu životne sredine, rudarstva i prostornog planiranja Republike Srbije.

LITERATURA

[1] S. Šerbula, Kvalite vazduha u Boru i okolni, XVII Naučno stručni skup Ekološka istina 09, br. 17, 2009, str. 9–16.

[2] Analiza stanja životne sredine od šteta nastalih kao posledica prethodnog rada RTB Bor – Finalni izveštaj: Environmental resources management, The privatization agency of Serbia (RTB BOR EA), 2006, http:// //www.digitalnizavicaj.com/pdf/texts/170/Analiza_stanja_zivotne_sredine_Borpdf.pdf

[3] M. Bugarin, Radojka Jonović, Ljiljana Avramović, Tretman industrijskih otpadnih voda iz procesa proizvodnje bakra u RTB Bor, Rudarski radovi **4** (2012) 1–8.

[4] J. Sokolović, R. Stanojlović, S. Barbulović, Z.S. Marković, Z. Štirbanović, Analiza stanja zagađenosti životne sredine u RTB-u Boru, XV naučno-stručni skup Ekološka istina, 2007, Kniga radova, str. 174–180.

[5] Lj. Obradović, M. Bugarin, V. Marinković, Uticaj rudničkih objekata RTB Bor na zagađenje okolnih površinskih vodotokova, Rudarski radovi **4** (2012) 185–196.

[6] Project for Management of mining waste tailing damp at the region Bor, Duration: August 2011 – August 2013.

[7] D.B. Johnson, K.B. Hallberg, Acid mine drainage remediation options: a review, Sci. Total Environ. **338** (2005) 3–14.

[8] A.J. Gunson, B. Klein, M. Veiga, S. Dunbar, Reducing mine water requirements, J. Clean. Prod. **21** (2012) 71–82.

[9] A.O. Fadiran, C.L. Dlamini, J.M. Thwala, Environmental Assessment of Acid Mine Drainage Pollution on Surface Water Bodies around Ngwenya Mine, Swaziland, J. Environ. Prot. **5** (2014) 164–173.

[10] N.F. Gray, Environmental impact and remediation of acid mine drainage: a management problem, Environ. Geol. **30** (1997) 62–71.

[11] C.L. Dlamini, A.O. Fadiran, J.M. Thwala, A Study of Environmental Assessment of Acid Mine Drainage in Ngwenya, Swaziland, J. Environ. Prot. **4** (2013) 20–26.

[12] B. Dalmacija, I. Ivančev-Tumbas, Analiza vode-kontrola kvaliteta, tumačenje rezultata, Prirodno matematički fakultet, Novi Sad, 2004.

[13] V. Gvozdić, J. Brana, D. Puntarić, D. Vidosavljević, D. Roland, Changes in the Lower Drava River Water Quality Parameters Over 24 Years, Arh. Hig. Rada Toksikol. **62** (2011) 325–323.

[14] Council DIRECTIVE 79/869/EEC of 09 October 1979 concerning the method of measurement and frequencies of sampling and analysis surface water intended for the abstraction of drinking water in Member States.

[15] Pravilnik o opasnim materijama u vodama („Sl. Glasnik SRS“, br. 31/82).

[16] Directive 2006/11/EC of the European Parliament and of the Council of 15 February 2006 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community.

[17] Council Directive 75/440/EEC of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in Member States.

SUMMARY

IMPACT ASSESSMENT OF MINE DRAINAGE WATER AND MUNICIPAL WASTEWATER ON THE SURFACE WATER NEAR THE CITY OF BOR

Vojka R. Gardić[1], Jelena V. Petrović[1], Lidija V. Đurđevac-Ignjatovic[1], Srđan R. Kolaković[2], Svetlana R. Vujović[2]

[1]*Mining and Metallurgy Institute Bor, Bor, Serbia*
[2]*Faculty of Technical Sciences, Department of Civil Engineering, University of Novi Sad, Novi Sad, Serbia*

(Professional paper)

Mining and copper production in Bor, in the past hundred years, had a huge impact on the environment of town, but also in a wide region. In the area of Bor, in the zone of Mining and Smelting Company (RTB) activity, over 29,000 ha of land under forests and fields is degraded. The area of degraded agricultural land in the Bor municipality is over 60% of total agricultural land. Wastewater, generated in the sites of RTB Bor, pollutes the Bor River and Krivelj River, which further flow into the Timok River and Danube River. These pollutions are often presented by low pH value, increased content of heavy metal ions, suspended particles and fine particles of flotation tailings, which are deposited in the valleys of these rivers on the area of over 2000 hectares. During the decades of exploitation of ore from the open pit Bor at different locations (“Visoki Planir” – also called “Oštreljski planir”, “Severni planir” dump of ore body “H” (RTH)) gangue and tailings were delayed. The largest amount of tailings, about 150 million tons, was postponed on location Visoki planir. The effect of the mining waste and the impact of the whole process of processing copper ore to the final products on the environment, was conducted during the 4th study period of the project “*Management of mining waste-tailing dump in the Bor region*”, supported by the Japan Society for the Promotion Science and the Japan international cooperation Agency and the Ministry of environment, Mining and Spatial planning of the Republic of Serbia. Influence of season on the level of pollutants in soil and water, the impact on water quality in the river Timok and the River Danube, was conducted during first three periods of project. This paper presents the results of the third study period. The third period of research, which was conducted over a period of 17.10.2012 to 17.01.2013, included a review of pollution sources and define their impact on the environment. The study included the following sources of pollution: mining waste and drainage water originating from the active mine (Bor pit, field 1 Krivelj large tailings, flotation tailings in Bor RTH, metallurgical water), as well as the drainage water from the flotation tailings, which are no longer in operation (field 2 flotation tailings Great Krivelj, drainage water from the old Bor flotation tailings), the old inactive landfill mine gangue (Saraka landfill, Veliki planir – tailings from the old Bor mine, landfill mine gangue from mine RTH) and the city – urban waste water, which are discharged without treatment directly into the watercourse Bor River. wastewater directly pollutes Bor River and Krivalj River.

Keywords: Mining • Wastewater • Surface water • Chemical characterization

Uticaj kvaliteta semena na oksidativnu stabilnost hladno presovanog ulja suncokreta

Etelka B. Dimić[1], **Tamara Đ. Premović**[1], **Aleksandar A. Takači**[1], **Vesna B. Vujasinović**[2], **Olgica F. Radočaj**[1], **Sanja B. Dimić**[1]

[1]*Univerzitet u Novom Sadu, Tehnološki fakultet, Bulevar cara Lazara 1, 21000 Novi Sad, Srbija*
[2]*Visoka škola za menadžment i poslovne komunikacije, Mitropolita Stratimirovića 110, 21205 Sremski Karlovci, Srbija*

Izvod

U ovom radu ispitivan je uticaj vremena skladištenja, sadržaja nečistoće i ljuske u masi semena na oksidativnu stabilnost hladno presovanog ulja suncokreta. Oksidativna stabilnost ulja je analizirana primenom *RSM* metodologije (*Response Surface Methodology*), određivanjem produkata oksidacije izraženih preko vrednosti specifičnih apsorbancija na 232 nm za konjugovane diene i 270 nm za konjugovane triene, u svežem ulju i u ulju nakon temperiranja u uslovima Schaal–Oven testa (96 h pri 63±2 °C). Utvrđeno je da vreme skladištenja semena značajno utiče na sadržaj primarnih ($A^{1\%}_{232\text{nm}}$) i sekundarnih ($A^{1\%}_{270\text{nm}}$) produkata oksidacije, kako pre (b_1–$p < 0{,}001$; b_1–$p = 0{,}021$) tako i nakon zagrevanja (b_1–$p = 0{,}048$; b_1–$p = 0{,}033$) ulja. Prisustvo različitog sadržaja nečistoće i ljuske semena u materijalu za presovanje takođe utiče na sadržaj konjugovanih diena i triena, ali njihov uticaj nije statistički značajan ni u svežem, ni u temperiranom ulju. Osim toga, izvestan efekat na vrednosti navedenih parametara kvaliteta ulja ispoljava i interakcije vremena skladištenja semena, sadržaja nečistoće i ljuske, kako kod svežeg ulja tako i kod ulja nakon termičke obrade, međutim ni njihov uticaj nije statistički značajan. Dobijeni rezultati su ukazali na činjenicu da hladno presovano ulje suncokreta pri umerenim temperaturama, do 63±2 °C, pokazuje dobru oksidativnu stabilnost.

Ključne reči: hladno presovano ulje suncokreta, oksidativna stabilnost, specifične apsorbancije, vreme skladištenja, sadržaj nečistoće i ljuske, *RSM.*

Dostupno na Internetu sa adrese časopisa: http://www.ache.org.rs/HI/

NAUČNI RAD

UDK 665.334.9:544:66

Hem. Ind. **69** (2) 175–184 (2015)

doi: 10.2298/HEMIND140216032D

Jestiva nerafinisana ulja se proizvode u malim pogonima tzv. mini-uljarama na uređajima malog ili srednjeg kapaciteta preradom sirovine iz regionalnog okruženja i primenom vrlo jednostavne tehnologije. To podrazumeva dobijanje ulja iz semena isključivo mehaničkim ceđenjem, najčešće na pužnoj presi. Po završenom presovanju ulja se dodatno prečišćavaju jedino sedimentacijom i/ili filtracijom, dok naknadno poboljšanje kvaliteta ulja primenom procesa rafinacije nije dozvoljeno [1–3]. Poslednjih godina tehnološki razvoj je omogućio ekonomski opravdanu proizvodnju jestivih nerafinisanih ulja [4,5], čiji je kvalitet određen, pre svega, izborom i kvalitetom sirovine. Međutim, proizvodnja ovih ulja posebno visokog kvaliteta smatra se pravim izazovom za proizvođače [6].

Pri određivanju sveobuhvatnog kvaliteta hladno presovanih ulja veoma je važno odrediti i oksidativnu stabilnost. Naime, procesi oksidacije biljnih ulja su neizbežni i oni nastupaju određenom dinamikom prvenstveno u zavisnosti od sastava ulja i prisutva sastojaka koji ubrzavaju ili usporavaju ove reakcije [7]. Kvarenje biljnih ulja uzrokovano oksidativnim procesima je najčešći tip kvarenja, a predstavlja proces oksidacije nezasićenog ugljovodoničnog lanca masnih kiselina. Tokom oksidacije lipida dolazi do promene hemijskog sastava i pogoršanja senzornog i nutritivnog kvaliteta ulja.

Oksidativna stabilnost ulja predstavlja vreme za koje se ona mogu sačuvati od intenzivnog procesa autooksidacije. Poznavanje stabilnosti ili održivosti biljnih ulja je važno kako bi se moglo unapred odrediti vreme čuvanja bez bitnih promena kvaliteta [2]. Ne retko se kod presovanih ulja beleže velike razlike upravo u oksidativnoj stabilnosti, o čemu govore i rezultati ispitivanja Matthäus i Brühl [6]. Prema njihovom mišljenju razlog za to su velika variranja u kvalitetu polaznog materijala korišćenog za proizvodnju ulja.

Na kvalitet ulja dobijenog hladnim presovanjem pored kvaliteta polazne sirovine i drugi parametri imaju značajan uticaj. Neki od mnogobrojnih faktora kvaliteta hladno presovanih ulja su: tehnološki parametri procesa prerade, vreme i uslovi čuvanja semena pre prerade, prisustvo nečistoće, kao i ljuske u materijalu za presovanje i dr. [2,8].

Schaal–Oven test je jedna od najjednostavnijih metoda ispitivanja oksidativnih promena ulja, čiji su uslovi najpribližniji realnim uslovima njihovog čuvanja [9,10]. Međutim, u novijoj literaturi se ipak retko nalaze rezultati koji se odnose na praćenje promena prime-

---

Prepiska: E.B. Dimić, Tehnološki fakultet, Bulevar cara Lazara 1, 21000 Novi Sad Srbija.
E-pošta: edimic@uns.ac.rs

Rad primljen: 16. februar, 2014
Rad prihvaćen: 7. april, 2014

nom Schaal-Oven testa kod hladno presovanih ulja suncokreta, iako postoji veliko opravdanje za to, budući da se ova ulja pretežno konzumiraju kao salatna ulja ili komponente određenih jela, koja nisu toplotno obrađena.

Oksidativni kvalitet je veoma važan segment proizvođačkog, ali i potrošačkog kvaliteta ulja, budući da utiče i na druge parametre, kao što su senzorni, nutritivni i hemijski kvalitet. S obzirom na to da hladno presovana ulja suncokreta spadaju u najrasprostranjenija semenska ulja na domaćem i inostranom tržištu, u ovom radu je analiziran pojedinačan i istovremeni uticaj dužine skladištenja semena, sadržaja nečistoće i sadržaja ljuske prisutnih u masi semena na oksidativnu stabilnost ulja dobijenih presovanjem na pužnoj presi. Oksidativne promene su ispitivane na osnovu koncentracije primarnih i sekundarnih proizvoda oksidacije izraženih preko vrednosti apsorbancija ulja u UV oblasti spektra pri talasnoj dužini od 232 i 270 nm.

EKSPERIMENTALNI DEO

Materijal

Za pripremanje uzoraka ulja korišćeno je seme suncokreta (*Helianthus annuus* L.) domaćeg hibrida CEPKO, čiji je sadržaj ulja iznosio 40,9±0,26%, sa dominantnom linolnom (55,61%) masnom kiselinom u sastavu ulja. Seme suncokreta je bilo proizvedeno u masovnoj proizvodnji na sirovinskom području za uzgoj suncokreta u AP Vojvodina i pre presovanja skladišteno je u silo ćeliji kapaciteta 14 tona pod uslovima niske temperature i dobre ventilacije tokom 0, 6 i 12 meseci.

Priprema materijala za presovanje i proces presovanja

Uzorci hladno presovanog ulja su proizvedeni na tradicionalan način u pogonu mini-uljare na opremi koja obuhvata čistilicu, ljuštilicu i pužnu presu. Ljuštenje semena je izvršeno na ljuštilici sa uklanjanjem ljuske strujom vazduha i gravitacijom. Rezultujuća mešavina ljuske, jezgra i neoljuštenog semena je razdvojena, ljuska je uklonjena strujom vazduha, dok je oljušteno jezgro odvojeno od neoljuštenog i slomljenog semena gravitacijom.

Ulja su proizvedena presovanjem oljuštenih jezgara, nakon različitog vremena skladištenja semena, sa dodatkom određenog sadržaja nečistoće i ljuske prema eksperimentalnom dizajnu (tabela 1).

Nečistoća koja je korišćena u eksperimentima je pretežno sadržala materije organskog porekla, uobičajeno prisutne u masi semena posle žetve (suvi delovi različitih biljaka, stablo, list, razna semena i dr.).

Uzorci su presovani korišćenjem svaki put po 5 kg pripremljenog materijala. Presovanje je obavljeno na pužnoj presi ("Anton Fries", Nemačka), kapaciteta 6–9 kg h^{-1}, i brzinom obrtaja puža 30–45 o/min. Temperatura ulja na izlazu iz prese iznosila je 50–60 °C. Ispresovana ulja su držana na sobnoj temperaturi (20–25 °C) u toku 24 časa radi sedimentacije rezidua, potom je gornji sloj dekantiran i filtriran kroz laboratorijski filter papir. Uzorci ulja su do analiza čuvani u tamnim staklenim bocama u frižideru pri temperaturi od 4 °C.

Tabela 1. Stvarne i kodirane vrednosti nezavisno promenljivih u eksperimentalnom dizajnu i rezultati specifičnih apsorbancija svežih uzoraka hladno presovanih ulja suncokreta; rezultati predstavljaju srednju vrednost tri određivanja ± SD; različita mala slova u kolonama ukazuju na postojanje statistički značajnih razlika ($p < 0,05$)
Table 1. Actual and coded values of variables in the experimental design with the response values (specific absorbance of the fresh and heated cold-pressed sunflower oil samples)

Oznaka uzorka	Vreme skladištenja, mesec		Sadržaj nečistoće, %		Sadržaj ljuske, %		Y_1	Y_2
	A	B	A	B	A	B	$A^{1\%}_{232nm}$	$A^{1\%}_{270nm}$
1	0	-1	0	-1	16	0	$0,96\pm0,05^{aaa}$	$0,16\pm0,02^{aaa}$
2	0	-1	5	0	0	-1	$0,92\pm0,03^{aba}$	$0,16\pm0,01^{aaa}$
3	0	-1	5	0	32	+1	$0,73\pm0,03^{aba}$	$0,20\pm0,02^{aaa}$
4	0	-1	10	+1	16	0	$0,68\pm0,04^{aaba}$	$0,24\pm0,03^{aaa}$
5[a]	0	-	0	-	0	-	$0,77\pm0,02^{aaa}$	$0,14\pm0,01^{aaa}$
6	6	0	0	-1	0	-1	$2,61\pm0,00^{aba}$	$0,34\pm0,00^{baa}$
7	6	0	0	-1	32	+1	$2,54\pm0,01^{aba}$	$0,41\pm0,00^{baa}$
8	6	0	5	0	16	0	$2,53\pm0,00^{abba}$	$0,39\pm0,00^{baa}$
9	6	0	10	+1	0	-1	$2,23\pm0,00^{aba}$	$0,23\pm0,00^{baa}$
10	6	0	10	+1	32	+1	$1,81\pm0,00^{ababa}$	$0,21\pm0,00^{baa}$
11	12	+1	0	-1	16	0	$1,32\pm0,02^{aba}$	$0,29\pm0,02^{baa}$
12	12	+1	5	0	0	-1	$1,36\pm0,02^{aba}$	$0,27\pm0,04^{baa}$
13	12	+1	5	0	32	+1	$0,65\pm0,01^{aba}$	$0,35\pm0,01^{baa}$
14	12	+1	10	+1	16	0	$1,43\pm0,03^{aaba}$	$0,38\pm0,03^{baa}$
15[a]	12	-	10	-	32	-	$1,12\pm0,03^{aaba}$	$0,42\pm0,02^{baa}$

[a] Uzorci ulja koji su dodati u istraživanja, A – stvarna vrednost, B – kodirana vrednost

Metode

Uvid u oksidativnu stabilnost ulja dobijen je na bazi apsorbancija svežih ulja, odnosno, ulja nakon temperiranja u uslovima Schaal–Oven testa (50 ml ulja u otvorenoj staklenoj posudi zagrevano je 96 h pri temperaturi od 63±2 °C) [11]. Metoda se sastoji u spektrofotometrijskom određivanju apsorbancije rastvora ulja u cikloheksanu u kiveti prečnika 1cm pri talasnim dužinama od 232 i 270 nm i izračunavanju apsorbancije za koncentraciju od 1 g ulja u 100 ml rastvora, $A^{1\%}_{232\text{nm}}$ i $A^{1\%}_{270\text{nm}}$. Iz odnosa ove dve vrednosti izračunata je *R*-vrednost, koja se smatra pouzdanim pokazateljem kvaliteta nerafinisanih ulja [12]:

$$R\text{-vrednost} = A^{1\%}_{232\text{nm}}/A^{1\%}_{270\text{nm}}$$

Eksperimentalni dizajn i statistička analiza

RSM metoda je primenjena da bi se ispitao uticaj sastava materijala za presovanje na oksidativnu stabilnost uzoraka ulja. Nezavisno promenljivi faktori bili su: vreme skladištenja, sadržaj nečistoće i sadržaj ljuske. Primenjen je trofaktorski plan eksperimenata na tri nivoa po Box–Behnken dizajnu (BBD) ($\alpha = 3$) [13] koji predviđa 13 nezavisnih merenja. Stvarne i kodirane vrednosti prediktora su prikazane u tabeli 1, za sve uzorke, izuzev za uzorak broj 5 i 15, koji nisu bili obuhvaćeni BBD dizajnom. To su ulja koja su, prema nepublikovanim rezultatima naših prethodnih istraživanja, dodata u ova istraživanja, budući da imaju minimalne (uzorak broj 5) i maksimalne vrednosti (uzorak broj 15) ispitivanih nezavisno promenljivih. Zavisno promenljive su bile vrednosti specifičnih apsorbancija na 232 i 270 nm kao odzivi modela, i to u svežim uljima, kao i u uljima nakon temperiranja u uslovima Schaal–Oven testa. RSM metoda je primenjena na eksperimentalno dobijenim rezultatima primenom komercijalnog statističkog paketa Statistica 12. Eksperimentalno dobijeni podaci su predstavljeni kao model jednačine drugog stepena na bazi matematičkog modela, koji je prikazan jednačinom (1):

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

gde je: Y_i odziv; X_1 vreme skladištenja semena (mesec); X_2 sadržaj nečistoće (%); X_3 sadržaj ljuske (%); a b_0, b_1, b_2, b_3, b_{11}, b_{22}, b_{33}, b_{12}, b_{13} i b_{23} su regresioni koeficijenti.

Višestruka regresiona analiza je sprovedena primenom istog softvera koji je korišćen i za eksperimentalni dizajn. Podaci su predstavljeni u obliku srednje vrednosti ± standardna devijacija (*SD*). Statistička značajnost koeficijenata u regresionoj jednačini je ispitivana pomoću analize varijanse, *ANOVA* metode ($p < 0,05$), za svaki odziv. Za dodatnu proveru modela korišćen je koeficijent determinacije (R^2). Jednofaktorijalna analiza varijanse (ANOVA) i Tukey test primenjeni su radi određivanja statistički značajne razlike između rezultata ($p < 0,05$). Trodimenzioni grafici odzivnih površina su korišćeni da bi se objasnio efekat nezavisno promenljivih-prediktora (X_1, X_2 i X_3) na vrednosti odzivnih varijabli (Y_1–Y_4).

REZULTATI I DISKUSIJA

Uticaj vremena skladištenja, sadržaja nečistoće i ljuske semena na oksidativnu stabilnost svežih uzoraka hladno presovanih ulja suncokreta

Eksperimentalni dizajn, kao i vrednosti specifičnih apsorbancija, $A^{1\%}_{232\text{nm}}$ i $A^{1\%}_{270\text{nm}}$, dobijeni analizom sveže proizvedenih uzoraka ulja prikazani su u tabeli 1.

Kako specifične apsorbancije ukazuju na oksidativne promene na nezasićenim masnim kiselinama, cilj primenjenog modela je bio postizanje njihovih minimalnih vrednosti. Redukovan regresioni model predstavljen u jednačini (2) i (3) omogućava predviđanje uticaja nezavisno promenljivih faktora na vrednosti specifičnih apsorbancija, Y_1 na 232 nm i Y_2 na 270 nm:

$$Y_1 = 0{,}951 + 0{,}492X_1 - 0{,}038X_1^2 - 0{,}072X_2 + 0{,}003X_2^2 + 0{,}023X_3 - 0{,}001X_3^2 + 0{,}004X_1X_2 - 0{,}001X_1X_3 \quad (2)$$

$$Y_2 = 0{,}180 + 0{,}029X_1 - 0{,}002X_1^2 + 0{,}009X_2 - 0{,}002X_2^2 + 0{,}004X_3 + 0{,}001X_1X_2 \quad (3)$$

Primenjenim regresionim modelom za predviđanje vrednosti $A^{1\%}_{232\text{nm}}$ može se objasniti 96% postignutih rezultata, dok se primenjenim modelom za predviđanje vrednosti $A^{1\%}_{270\text{nm}}$ objašnjava 91% postignutih rezultata (tabela 2).

F-vrednost, koja iznosi 13,44 sa ukupnim $p = 0,005$, ukazuje da je primenjeni model odgovarajući i da obezbeđuje dobro slaganje između predviđenih i postignutih vrednosti sadržaja primarnih produkata oksidacije, izraženih apsorbancijom na 232 nm. *F*-vrednost, od 5,40 sa vrednošću $p = 0,039$, ukazuje da je model primenjen kod sekundarnih produkata oksidacije, izraženih apsorbancijom na 270 nm, takođe odgovarajući (tabela 2). Potvrda efikasnosti primenjenog modela su i predviđene vrednosti ispitivanih parametara kvaliteta, koje u odnosu na izmerene vrednosti najmanje odstupaju u uzorku 12 (odstupanje iznosi 0,003) za $A^{1\%}_{232\text{nm}}$, odnosno, u uzorku 11 (odstupanje iznosi 0,019) za $A^{1\%}_{270\text{nm}}$. Rezultati sprovedenih istraživanja su pokazali da vreme skladištenja semena ima veoma značajan linearan i kvadratni uticaj na sadržaj primarnih produkata oksidacije (b_1–$p < 0,001$, b_{11}–$p < 0,001$, tabela 2), i značajan uticaj na sadržaj sekundarnih produkata oksidacije u ispitivanom ulju (b_1–$p = 0,021$, b_{11}–$p = 0,016$, tabela 2). Rezultati istraživanja Dimić i sar. [14] takođe potvrđuju značaj vremena skladištenja semena suncokreta na vrednosti specifičnih apsorbancija u UV oblasti spektra u ulju dobijenom postupkom presovanja na pužnoj presi. Isti autori su naveli da se produženjem vremena skladištenja semena (stan-

Tabela 2. Značajnost regresionog modela (F-vrednost i koeficijenti regresije) i efekat procesnih varijabli na oksidativnu stabilnost svežih uzoraka hladno presovanih ulja suncokreta; b_1, b_2, b_3 *– linearni regresioni koeficijenti;* b_{12}, b_{13}, b_{23} *– interakcioni regresioni koeficijenti;* b_{11}, b_{22}, b_{33} *– kvadratni regresioni koeficijenti;* R^2*-regresioni koeficijent modela*

Table 2. The significance of the regression model (F-values and regression coefficients) and effects of the process variables on oxidative stability of fresh cold-pressed sunflower oils

Koeficijenti regresije	Y_1 $A^{1\%}_{232nm}$	Verovatnoća (p-vrednost[a])	Y_2 $A^{1\%}_{270nm}$	Verovatnoća (p-vrednost[a])
b_0	0,951	0,005	0,180	0,004
b_1	0,492	<0,001	0,029	0,021
b_2	–0,072	0,590	0,009	0,429
b_3	0,023	0,293	0,004	0,515
b_{12}	0,004	0,352	0,001	0,634
b_{13}	–0,001	0,427	–	–
b_{23}	–	–	–	–
b_{11}	–0,038	<0,001	–0,002	0,016
b_{22}	0,003	0,965	–0,002	0,498
b_{33}	–0,001	0,326	–	0,664
F-vrednost modela	13,44	–	5,40	–
p-vrednost modela	0,005	–	0,039	–
R^2	0,96	–	0,91	–

[a] Značajnost od $p < 0,05$

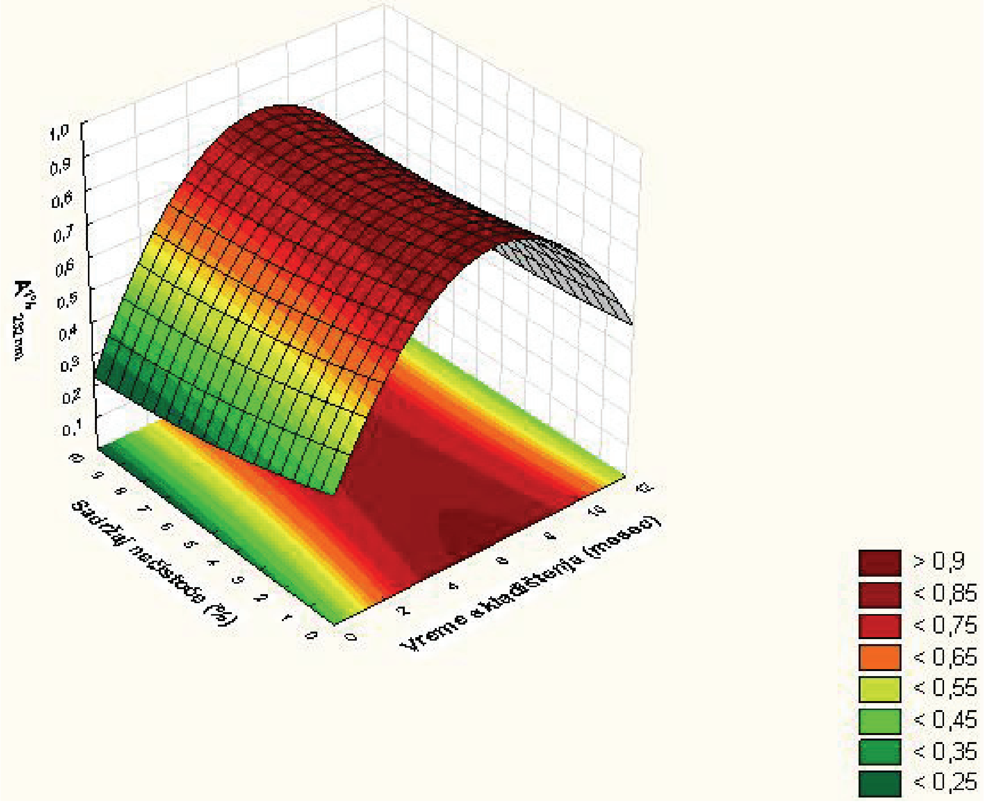
dardnog linolnog tipa) sa 3 na 12 meseci, održivost ulja smanjila za 9%, tj. da se sadržaj primarnih produkata oksidacije ulja povećao sa 2,10 na 2,30, a sekundarnih produkata oksidacije sa 0,22 na 0,34. Pri tome se *R*-vrednost ulja smanjila sa 9,55 na 6,77. U ulju oleinskog tipa takođe je uočeno smanjenje održivosti, odnosno povećanje sadržaja primarnih i sekundarnih produkata oksidacije, koje je čak bilo većeg intenziteta nego kod ulja linolnog tipa, budući da je iznosilo oko 20 do 35%.

Poznato je da se prisustvo nečistoće i ljuske semena nepovoljno odražava na kvalitet ulja, budući da može prouzrokovati povećanje sadržaja primarnih i sekundarnih produkata oksidacije [2]. U literaturi se, međutim, sreću različita mišljenja o uticaju prisutne ljuske semena. Tako, Subramaniam i sar. [15] navode da ljuštenje semena suncokreta utiče na smanjenje vrednosti specifičnih apsorbancija presovanog ulja na 232 i 270 nm, za oko 7 puta, sa 30,8 na 4,30 ($A^{1\%}_{232nm}$) i sa 1,60 na 0,225 ($A^{1\%}_{270nm}$). Rezultati Šmit i sar. [16] ukazuju da i delimično ljuštenje semena suncokreta takođe dovodi do smanjenja vrednosti apsorbancija na 232 i 270 nm, kao i povećanje *R*-vrednosti u proizvedenom hladno presovanom ulju (sa 6,34 na 7,40). Međutim, rezultati naših istraživanja pokazuju da dodata nečistoća i ljuska u materijalu za presovanje, iako je bilo za očekivati, ne ispoljavaju značajan uticaj na vrednosti ovih specifičnih apsorbancija u ispitivanom ulju. Vrednosti *p,* prikazane u tabeli 2, ukazuju da ni međusobne interakcije ispitivanih varijabli takođe nemaju značajan uticaj na oksidativnu stabilnost ulja (slike 1–3).

Vrednosti specifičnih apsorbancija sveže presovanih uzoraka ulja na 232 nm-Y_1 kreću se u opsegu od 0,65±0,01 (uzorak 13) do 2,61±0,00 (uzorak 6), dok su rezultati specifičnih apsorbancija na 270 nm-Y_2 u granicama od 0,14±0,01 (uzorak 5) do 0,42±0,02 (uzorak 15, tabela 1). *R*-vrednosti u analiziranom sveže proizvedenom ulju nalaze se u intervalu od 1,86±0,03 (uzorak 13) do 9,66±0,03 (uzorak 9).

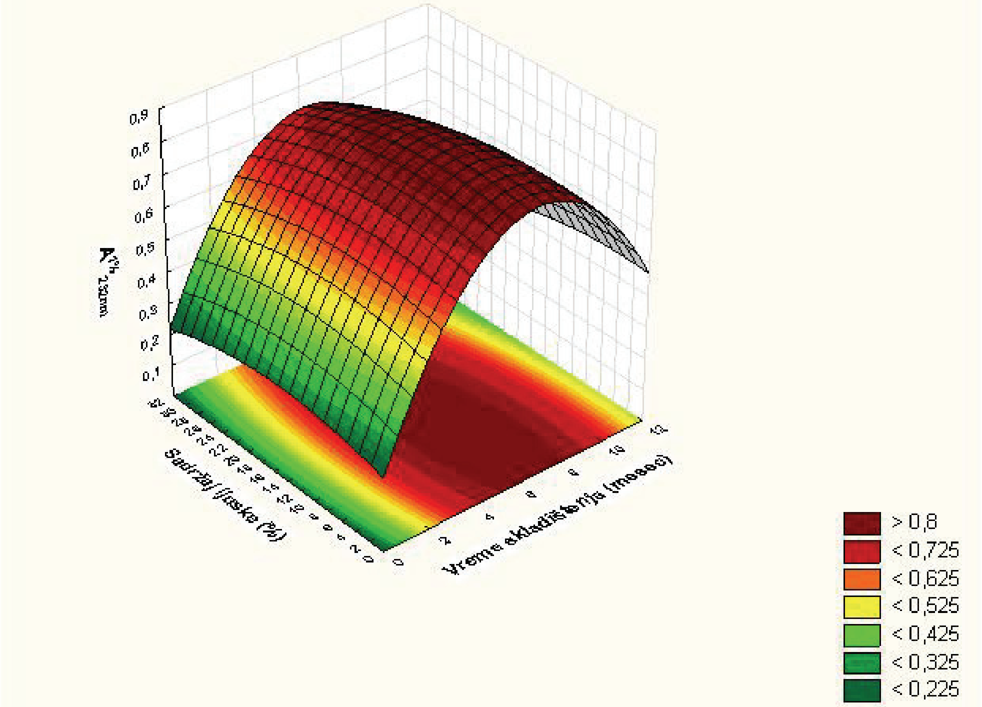
Zakonskim propisima vrednosti specifičnih apsorbancija nisu uključene u kriterijume kvaliteta, ali se smatra da nerafinisano ulje suncokreta dobrog kvaliteta treba da ima vrednost $A^{1\%}_{270nm}$ manju od 0,5 [17]. Ispitivana ulja su, prema vrednosti ovog parametra kvaliteta, ulja dobre oksidativne stabilnosti. Svega dva uzorka imaju vrednosti veće od 0,40 i to, uzorak 15 (0,42±0,02) i uzorak 7 (0,41±0,00). Razlog ovome je činjenica da je skladišteno očišćeno seme, a nečistoće su dodate u semenu masu neposredno pre presovanja. Grupa autora, Martin-Polvillo i sar. [7], su za ulje suncokreta linolnog tipa objavili vrednost od 0,46, a za ulje oleinskog tipa 0,42.

Dobijene vrednosti ispitivanih parametara su u skladu sa podacima iz literature za hladno ceđena ulja suncokreta sa domaćeg tržišta [2]. Za hladno ceđena ulja sa inostranog tržišta utvrđeno je da vrednosti apsorbancija na 232 nm variraju u opsegu od 0,92 do 0,99, a na 270 nm, od 0,08 do 0,1, dok su *R*-vrednosti u opsegu od 9,70 do 11,50 [18,19]. U hladno presovanom ulju oleinskog tipa sadržaj primarnih i sekundarnih produkta oksidacije iznosi, 1,94 ($A^{1\%}_{232nm}$) i 0,16 ($A^{1\%}_{270nm}$), a *R*-vrednost iznosi 12 [10]. U rafinisanom suncokretovom ulju specifične apsorbancije na 232 nm imaju vrednost od 1,28 do 1,52, na 270 nm, od 0,26 do 1,35, dok je *R*-vrednost u intervalu od 1,05 do 4,97 [20]. U nerafinisanom ulju, ekstrahovanom heksanom, $A^{1\%}_{232nm}$ iznosi 3,28, $A^{1\%}_{270nm}$, 0,82, a *R*-vrednost 4 [21].



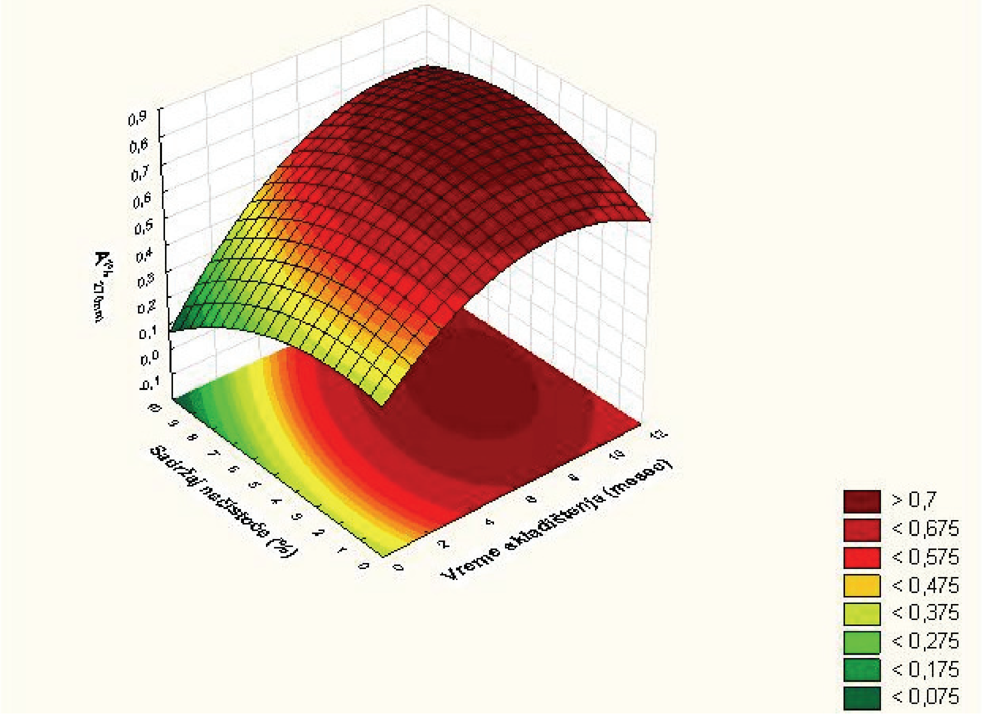
Slika 1. Odzivna površina vrednosti specifičnih apsorbancija na 232 nm ($A^{1\%}_{232nm}$) hladno presovanih ulja suncokreta u zavisnosti od vremena skladištenja i sadržaja nečistoće.

Figure 1. Response surface for the specific absorbance of the cold-pressed sunflower oil samples in relation to the storage time and the impurities content measured at 232 nm ($A^{1\%}_{232nm}$).



Slika 2. Odzivna površina vrednosti specifičnih apsorbancija na 232 nm ($A^{1\%}_{232nm}$) hladno presovanih ulja suncokreta u zavisnosti od vremena skladištenja i sadržaja ljuske.

Figure 2. Response surface for the specific absorbance of the cold-pressed sunflower oil samples in relation to the storage time and the husk content measured at 232 nm ($A^{1\%}_{232nm}$).



Slika 3. Odzivna površina vrednosti specifičnih apsorbancija na 270 nm ($A^{1\%}_{270nm}$) hladno presovanih ulja suncokreta u zavisnosti od vremena skladištenja i sadržaja nečistoće.
Figure 3. Response surface diagram for specific absorbance of the cold-pressed sunflower oil samples in relation to the storage time and the impurities content measured at 270 nm ($A^{1\%}_{270nm}$).

Prema literaturnim podacima vrednosti $A^{1\%}_{232\text{nm}}$, $A^{1\%}_{270\text{nm}}$ i R-vrednost (respektivno) za različite vrste hladno presovanih ulja su sledeće: maslinovo ulje 4,21; 0,37; 11,38 [10], laneno ulje 1,47–3,10; 0,34–0,56; 3,97–6,59 [2], orahovo ulje 1,04–1,21; 0,05–0,07; 17,3–20,8 [22] i tikvino ulje 1,96–4,11; 0,12–0,54; 8,23–13,46 [23]. Po rezultatima Pawlowicz i sar. [24] vrednosti apsorbancija $A^{1\%}_{1\text{cm}}$ pri 232 nm kod hladno presovanog repičinog ulja različitih sorti su se kretale od 0,19 do 2,08, a $A^{1\%}_{1\text{cm}}$ pri 268 nm od 0,05 do 0,3.

Uticaj vremena skladištenja semena, sadržaja nečistoće i ljuske na oksidativnu stabilnost hladno presovanog ulja suncokreta pri uslovima Schaal–Oven testa

Određivanje oksidativne stabilnosti Schaal-Oven testom je sprovedeno temperiranjem ulja pri 63 °C u kontaktu sa vazduhom, u periodu od 4 dana. Nakon isteka ovog vremena u ulju su utvrđene oksidativne promene određivanjem vrednosti specifičnih apsorbancija pri talasnim dužinama 232 i 270 nm. Rezultati su prikazani u tabeli 3, a značajnost regresionog modela (F-vrednost) i efekat procesnih varijabli na oksidativnu stabilnost uzoraka ulja pri uslovima Schaal-Oven testa su dati u tabeli 4.

Cilj primenjenog modela je i u ovom slučaju bio postizanje minimalnih vrednosti specifičnih apsorbancija temperiranog ulja. Analiza varijanse pokazuje da je primenjeni regresioni model odgovarajući, sa vrednostima, $F = 5{,}55$, $R^2 = 0{,}94$ i $p = 0{,}048$ za apsorbanciju Y_3–$A^{1\%}_{232\text{nm}}$, odnosno sa vrednostima, $F = 5{,}76$, $R^2 = 0{,}95$ i $p = 0{,}034$ za apsorbanciju Y_4 $A^{1\%}_{270\text{nm}}$ (tabela 4). Zavisnost između ispitivanih nezavisno promenljivih faktora i vrednosti specifičnih apsorbancija može se opisati jednačinama (4) i (5), koje slede:

$$Y_3 = 1{,}996 + 0{,}177X_1 - 0{,}007X_1^2 + 0{,}044X_2 - 0{,}002X_2^2 - 0{,}056X_3 + 0{,}001X_3^2 - 0{,}009X_1X_2 + 0{,}003X_1X_3 + 0{,}001X_2X_3 \quad (4)$$

$$Y_4 = 0{,}172 + 0{,}028\text{X}_1 - 0{,}001\text{X}_1^2 + 0{,}001X_2 + 0{,}001X_2^2 - 0{,}001X_3 + 1{,}218X_3^2 - 0{,}001X_1X_2 + 0{,}0002X_1X_3 - 8{,}758X_2X_3 \quad (5)$$

Potvrda efikasnosti primenjenog matematičkog modela su i predviđene vrednosti ispitivanih parametara, koje u odnosu na izmerene vrednosti najmanje odstupaju u uzorcima ulja oznake 11, za $A^{1\%}_{232\text{nm}}$ (odstupanje iznosi 0,037) i uzorka ulja oznake 14, za $A^{1\%}_{270\text{nm}}$ (odstupanje iznosi 0,002).

Utvrđeno je da linearan efekat dodate nečistoće, za razliku od linearnog efekta ljuske semena, doprinosi povećanju vrednosti specifičnih apsorbancija Y_3 i Y_4. Međutim, prisustvo različitog sadržaja nečistoće i ljuske semena u materijalu za presovanje nema značajan uticaj na sadržaj konjugovanih diena i triena u ulju nakon zagrevanja, što je takođe bio slučaj i u svežim uzorcima

Tabela 3. Stvarne i kodirane vrednosti nezavisno promenljivih u eksperimentalnom dizajnu i rezultati specifičnih apsorbancija uzoraka hladno presovanih ulja suncokreta u uslovima Schaal–Oven testa; rezultati predstavljaju srednju vrednost tri određivanja ± SD; različita mala slova u kolonama ukazuju na postojanje statistički značajnih razlika (p < 0,05)
Table 3. Actual and coded values of variables in the experimental design with the response values (specific absorbance of the cold-pressed sunflower oil samples under the Schaal–Oven's test conditions)

Oznaka uzorka	Vreme skladištenja, mesec		Sadržaj nečistoća, %		Sadržaj ljuske, %		Y_3	Y_4
	A	B	A	B	A	B	$A^{1\%}_{232nm}$	$A^{1\%}_{270nm}$
1	0	-1	0	-1	16	0	1,55±0,05^{aaa}	0,15±0,01^{aaa}
2	0	-1	5	0	0	-1	2,02±0,08^{aaa}	0,23±0,01^{aaa}
3	0	-1	5	0	32	+1	0,98±0,08^{aaa}	0,19±0,02^{aaa}
4	0	-1	10	+1	16	0	1,64±0,05^{aaa}	0,27±0,01^{aba}
5^{a}	0	-	0	-	0	-	2,08±0,10^{aaa}	0,14±0,01^{aaa}
6	6	0	0	-1	0	-1	2,50±0,00^{baa}	0,32±0,00^{baa}
7	6	0	0	-1	32	+1	2,45±0,00^{baa}	0,35±0,00^{baa}
8	6	0	5	0	16	0	2,42±0,00^{baa}	0,32±0,00^{baa}
9	6	0	10	+1	0	-1	2,49±0,00^{baa}	0,36±0,00^{bba}
10	6	0	10	+1	32	+1	2,39±0,00^{baa}	0,34±0,00^{bba}
11	12	+1	0	-1	16	0	2,97±0,01^{baa}	0,35±0,01^{baa}
12	12	+1	5	0	0	-1	3,01±0,02^{baa}	0,32±0,01^{baa}
13	12	+1	5	0	32	+1	3,03±0,02^{baa}	0,34±0,01^{baa}
14	12	+1	10	+1	16	0	1,80±0,04^{baa}	0,40±0,03^{bba}
15^{a}	12	-	10	-	32	-	2,68±0,01^{baa}	0,48±0,02^{bba}

[a] Uzorci ulja koji su dodati u istraživanja, A – stvarna vrednost, B – kodirana vrednost

Tabela 4. Značajnost regresionog modela (F-vrednost) i efekat procesnih varijabli na oksidativnu stabilnost uzoraka hladno presovanih ulja suncokreta pri uslovima Schaal–Oven testa; b_1, b_2, b_3 – linearni regresioni koeficijenti; b_{12}, b_{13}, b_{23} – interakcioni regresioni koeficijenti; b_{11}, b_{22}, b_{33}-kvadratni regresioni koeficijenti; R^2 – regresioni koeficijent modela
Table 4. The significance of the regression model (F-values and regression coefficients) and effects of the process variables on oxidative stability of the cold-pressed sunflower oil samples under the Schaal–Oven's test conditions

Koeficijenti regresije	Y_3 $A^{1\%}_{232nm}$	Verovatnoća (p-vrednost^{a})	Y_4 $A^{1\%}_{270nm}$	Verovatnoća (p-vrednost^{a})
b_0	1,996	<0,001	0,172	0,004
b_1	0,177	0,048	0,028	0,033
b_2	0,044	0,619	0,001	0,965
b_3	–0,056	0,083	–0,001	0,862
b_{12}	–0,009	0,134	–0,001	0,548
b_{13}	0,003	0,099	0,0002	0,397
b_{23}	0,001	0,833	–8,758	0,755
b_{11}	–0,007	0,258	–0,001	0,159
b_{22}	–0,002	0,771	0,001	0,394
b_{33}	0,001	0,351	1,218	0,917
F-vrednost modela	5,55	–	5,76	–
p-vrednost modela	0,048	–	0,034	–
R^2	0,94	–	0,95	–

[a] Značajnost od $p < 0{,}05$

ulja (bez zagrevanja). Interakcije vremena skladištenja semena, sadržaja nečistoće i ljuske, takođe ne ispoljavaju značajan efekat na vrednosti ovih parametara kvaliteta ulja (tabela 4).

Za razliku od faktora kvadrata vremena skladištenja, čiji uticaj na vrednosti $A^{1\%}_{232nm}$ i $A^{1\%}_{270nm}$ ulja nije bio značajan, linearan efekat vremena skladištenja semena značajno doprinosi povećanju vrednosti ovih parametara (b_1–p = 0,048, b_1–p = 0,033, tabela 4).

Vrednosti specifičnih apsorbancija na 232 nm u ulju nakon temperiranja kreću se u opsegu od 0,98±0,08 (uzorak 3) do 3,03±0,02 (uzorak 13), dok na 270 nm iznose od 0,14±0,01 (uzorak 5) do 0,48±0,02 (uzorak 15, tabela 3). R-vrednosti variraju u intervalu od

4,55±0,21 (uzorak 14) do 14,78±0,05 (uzorak 5). Prema podacima Romanić i Dimić [25], *R*-vrednost rafinisanog ulja suncokreta, temperiranog pri istim uslovima, je daleko manja i iznosi 2,72.

Upoređujući rezultate Schaal–Oven testa (tabela 3) u odnosu na sveže proizvedena ulja (tabela 1) može se konstatovati da zagrevanje ulja pri 63±2 °C u toku 96 h uzrokuje izvesne oksidativne promene, što se manifestuje povećanjem sadržaja konjugovanih diena (Y_3) i triena (Y_4) u odnosu na njihove početne vrednosti (Y_1 i Y_2). Najveće povećanje sadržaja konjugovanih diena-$A^{1\%}_{232\text{nm}}$ se uočava kod ulja proizvedenih iz semena skladištenog 12 meseci, dok su promene kod ulja proizvedenih iz semena skladištenog do 6 meseci (66,67% uzoraka) bile neznatne. Ova činjenica potvrđuje negativan uticaj dužeg vremena skladištenja semena pre prerade na oksidativnu stabilnost ulja. Međutim, vrednosti apsorbancije $A^{1\%}_{232\text{nm}}$ analiziranih ulja pre i nakon Schaal–Oven testa imaju izuzetno slabu linearnu međuzavisnost (R^2 = 0,11). Objašnjenje za to treba tražiti u kompleksnosti hemizma oksidacije ulja koji se odigrava pri zagrevanju i u kome učestvuju kako antioksidansi tako i prooksidansi poreklom iz ulja, a važnu ulogu imaju i reakcioni uslovi i brojni drugi činioci [26].

Za razlike u sadržaju oksidativnih produkata u ulju pre i nakon zagrevanja generalno se može reći da su neznatne. U sadržaju produkata u vidu konjugovanih diena, $A^{1\%}_{232\text{nm}}$, razlike kod 60% uzoraka su bez ili sa minimalnim promenama, kod 33% uzoraka vrednost $A^{1\%}_{232\text{nm}}$ je povećana za oko 1,5, a svega je kod jednog uzorka (uzorak 13) došlo do povećanja vrednosti sa razlikom većom od 2 (2,38).

Razlike u sadržaju oksidativnih produkata u vidu konjugovanih triena, $A^{1\%}_{270\text{nm}}$, pre i nakon zagrevanja ulja su takođe minimalne, iznose od 0,02 (uzorak 14) do 0,13 (uzorci 9 i 10). Ovi rezultati potvrđuju dobru oksidativnu stabilnost hladno presovanog ulja suncokreta pri umerenim temperaturama (60–65 °C). Naime, prema rezultatima Ramadan [27] vrednosti apsorbancije rafinisanog suncokretovog ulja linolnog tipa (61,1% linolne kiseline) pri 232 nm u uslovima Schaal–Oven testa (60±2 °C) je povećana sa oko 2 na 8, a pri 270 nm sa 0,5 na 3.

Između sadržaja konjugovanih triena u našim ispitivanim uzorcima pre i nakon temperiranja utvrđeno je postojanje linearne međuzavisnosti (R^2 = 0,645).

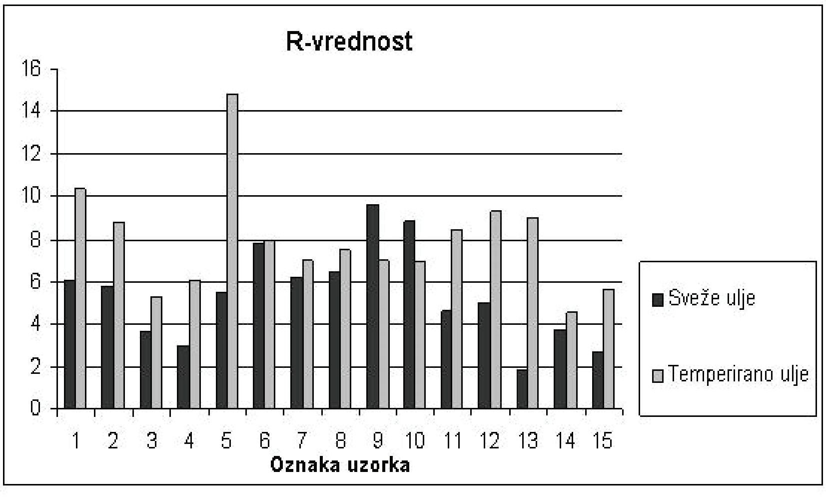
S obzirom na promene u vrednosti navedenih specifičnih apsorbancija tokom zagrevanja neminovno dolazi i do promena u *R*-vrednosti ispitivanih ulja. Na slici 4 su prikazane *R*-vrednosti uzoraka sveže presovanih i temperiranih ulja.

Kako navode Matijašević i Turkulov [17] nerafinisana ulja suncokreta dobrog kvaliteta treba da imaju R-vrednost najmanje 8. U tom pogledu može se reći da su od svih ispitanih uzoraka, kako sveže presovanih tako i temperiranih ulja, 10 ulja ispunjavaju navedeni kriterijum kvaliteta. Posebno treba istaći da je kod većine uzoraka (izuzev uzoraka 9 i 10) tokom zagrevanja došlo čak do povećanja *R*-vrednosti, što je rezultat neznatnih promena apsorbancija $A^{1\%}_{270\text{nm}}$. Ova činjenica, takođe, ukazuje na otpornost hladno presovanog ulja suncokreta prema oksidativnim promenama pri umerenim temperaturama.

ZAKLJUČAK

Ovim radom je prikazano da se sadržaj primarnih i sekundarnih produkata oksidacije hladno presovanog ulja suncokreta, izraženih pomoću specifičnih apsorbancija pri 232 i 270 nm, mogu dobro predvideti korišćenjem RSM metode.

Matematički modeli i grafička interpretacija su pokazali da je vreme skladištenja semena značajna varijabla za vrednosti sadržaja primarnih i sekundarnih pro-



Slika 4. R-vrednost hladno presovanih ulja suncokreta pre i posle zagrevanja pri uslovima Schaal–Oven testa.
Figure 4. R-values of the cold-pressed sunflower oil samples before and after heating under the Schaal–Oven's test conditions.

dukata oksidacije, kako svežeg tako i termički tretiranog ulja, kao odzive ovog modela.

Ustanovljeno je da prisustvo različitog sadržaja nečistoće i ljuske semena u materijalu za presovanje, utiče na sadržaj konjugovanih diena i triena u ulju i pre i nakon zagrevanja, ali da njihov uticaj nije statistički značajan. Interakcije vremena skladištenja semena, sadržaja nečistoće i ljuske u svežim uzorcima ulja, kao i kod ulja nakon temperiranja, takođe ispoljavaju efekat na vrednosti ovih parametara kvaliteta, koji međutim nije statistički značajan.

Zagrevanjem ulja pri uslovima Schaal-Oven testa nije došlo do izrazite promene oksidativnog kvaliteta, što ukazuje na činjenicu da je hladno ceđeno ulje suncokreta pri umerenim temperaturama, do 65 °C, pokazuje dobru stabilnost.

Zahvalnica

Autori se zahvaljuju Ministarstvu za nauku i tehnološki razvoj Republike Srbije za finansijsku podršku (projekat: TR 31014), a takođe i Miroslavu Đurka iz mini-uljare Vitastil-Erdevik, na ustupanju celokupnog neophodnog materijala, pribora i opreme, kao i pruženoj pomoći pri sprovođenju procesa presovanja.

LITERATURA

[1] A. De Leonardis, V. Macciola, A. Di Rocco, Oxidative stabilization of cold-pressed sunflower oil using phenolic compounds of the same seeds, J. Sci. Food Agric. **83** (2003) 523–528.

[2] E. Dimić, Hladno ceđena ulja, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, 2005.

[3] B. Matthäus, F. Spener, What we know and what we should know about virgin oils – a general introduction, Eur. J. Lipid Sci. Technol. **110** (2008) 597–601.

[4] A. Lanzani, P. Bondioli, A. Gasparoli, E. Fedeli, Nota tecnica sull'olio di girasole ottenuto per via umida: effetto di una parziale raffinazione sul colore e sulla conservabilit`a all'invecchiamento, Riv. Ital. Sost. Grasse **65** (1988) 493–496.

[5] M.M. Contini, M. Moresi, Oli di oliva e di girasole per spremitura meccanica senza fiscoli. Caratterizzazione chimico-fisica ed analisi costi/benefici, Industrie Alimentari **32** (1994) 149–156.

[6] B. Matthäus, L. Brühl, Why is it so difficult to produce high – quality virgin rapeseed oil for human consumption? Eur. J. Lipid Sci. Technol. **110** (2008) 611–617.

[7] M. Martin-Polvillo, G. Marquez-Ruiz, M. C. Dobarganes, Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature, J. Am. Oil Chem. Soc. **81** (2004) 577–583.

[8] M. Raß, C. Schein, B. Matthäus, Virgin sunflower oil, Eur. J. Lipid Sci. Technol. **110** (2008) 618–624.

[9] K. Warner, E. N. Frankel, T.L. Mounts, Flavor and oxidative stability of soybean, sunflower and low erucic acid rapeseed oils, J. Am. Oil Chem. Soc. **66** (1989) 558––564.

[10] E. Dimić, R. Romanić, Analiza kvaliteta maslinovog ulja i hladno ceđenog suncokretovog ulja oleinskog tipa, Uljarstvo **35** (2004) 17–26.

[11] J. Pokorny, J., S. Dobiasova, J. Davidek, Repeatability of the determination of oxidative stability of vegetable oils using the Schaal-Oven test, Scientific papers of the Prague Institute of Chemical Technology **58** (1985) 163––173.

[12] E. Dimić, J. Turkulov, Kontrola kvaliteta u tehnologiji jestivih ulja, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, 2000.

[13] R. Myers, H.D.C. Montgomery, C.M. Anderson, Response surface methodology: Process and product optimization using designed experiment, 3rd ed., Wiley, New York, 2009.

[14] E. Dimić, D. Škorić, R. Romanić, V. Dimić, Uticaj vremena čuvanja semena na kvalitet hladno ceđenog ulja suncokreta oleinskog i linolnog tipa, Uljarstvo **35** (2004) 5––10.

[15] R. Subramanian, M. Nakajima, T. Kimura, T. Maekawa, Membrane process for premium quality expeller-pressed vegetable oils, Food Res. International **31** (1998) 587–593.

[16] K. Šmit, E. Dimić, R. Romanić, K. Bjelobaba - Bošnjak, B. Mojsin, Uticaj ljuske na kvalitet hladno ceđenog ulja suncokreta, 46. Savetovanje: Proizvodnja i prerada uljarica, Zbornik radova, pp 89-93, Petrovac na moru, 2005.

[17] B.O. Matijašević, J. Turkulov, Tehnologija ulja i masti, I deo, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, 1980.

[18] A. De Leonardis, V. Mcciola, M. De Felice, Chemical and commercial characteristic of cold pressed sunflower seed oils, Italian Food and Beverage Technology **25** (2001) 46–52.

[19] A. De Leonardis, V. Macciola, N. Di Domenico, A first pilot study to produce a food antioxidant from sunflower seed shells (*Helianthus annuus*), Eur. J. Lipid Sci. Technol. **107** (2005) 220–227.

[20] E. Dimić, D. Tešanović, R. Romanić, V. Vukša, Promene kvaliteta jestivih rafinisanih ulja suncokreta tokom čuvanja, I Deo: Senzorni i hemijski kvalitet, Uljarstvo **34** (2003) 25–31.

[21] S. Latif, F. Anwar, Effect of aqueous enzymatic processes on sunflower oil quality, J. Am. Oil Chem. Soc. **86** (2009) 393–400.

[22] M.L. Martinez, D.M. Maestri, Oil chemical variation in walnut genotypes grown in Argentina. Eur. J. Lipid Sci. Technol. **110** (2008) 1183–1189.

[23] R. Rabrenović, Uticaj fizičko-hemijskih karakteristika semena uljane tikve (*Cucurbita pepo* L.) na kvalitet i nutritivna svojstva hladno presovanog ulja, Doktorska disertacija, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, 2011.

[24] R. Pawlowicz, J. Gromadzka, M. Tynek, R. Tylingo, W. Wardencki, Gy. Karlovits, The influence of the UV irradiation on degradation of virgin rapeseed oils, Eur. J. Lipid Sci. Technol. **115** (2013) 648–658.

[25] R. Romanić, E. Dimić, Oksidativna stabilnost hladno ceđenog ulja suncokreta oleinskog tipa pri povišenim temperaturama, Uljarstvo **41** (2010) 45–49.

[26] N.V. Yanishlieva, in: J. Pokorny, N. Yanishlieva, M. Gordon (Eds.), Antioxidants in Food, Woodhead Publishing Ltd, Cambridge, 2001, pp. 22–70.

[27] M.F. Ramadan, Healthy blends of high linoleic sunflower oil with selected cold pressed oils: Functionality, stability and antioxidative characteristics, Ind. Crop. Prod. **43** (2013) 65–73.

SUMMARY

EFFECT OF SEED QUALITY ON OXIDATIVE STABILITY OF COLD-PRESSED SUNFLOWER OIL

Etelka B. Dimić[1], Tamara Đ. Premović[1], Aleksandar A. Takači[1], Vesna B. Vujasinović[2], Olgica F. Radočaj[1], Sanja B. Dimić[1]

[1]University of Novi Sad, Faculty of Technology, Blvd cara Lazara 1, 21000, Novi Sad, Serbia

[2]College of Professional Studies in Management and Business Communication, Mitropolita Stratimirovića 110, 21205 Sremski Karlovci, Serbia

(Scientific paper)

The objective of this study was to investigate the impact of the storage time, content of the husk and impurities in the seed mass on the oxidative stability of the cold pressed sunflower oil. The oxidative stability of oil was analyzed by the Response Surface Methodology (RSM), where the response value (output) of the model was the content of oxidation products measured via specific absorbance at 232 nm for conjugated dienes and 270 nm for conjugated trienes. Analyses were performed on the fresh oil samples and oil samples tempered under the Schaal–Oven's test conditions (96 h at 63±2 °C). It was concluded that the seed storage time had significant impact on the primary ($A^{1\%}_{232\mathrm{nm}}$) and secondary ($A^{1\%}_{270\mathrm{nm}}$) oil oxidation products, both before (b_1–$p < 0.001$; b_1–$p = 0.021$) and after (b_1–$p = 0.048$; b_1–$p = 0.033$) the heating of oil. The content of impurities and husks in the seeds used for pressing also had an influence of the conjugated dienes and trienes contents in both, fresh and heated oil samples. However, their impact was not statistically significant. Interaction of seed storage time, the content of impurities and husks also exert some effect on the values of these quality parameters in the fresh oil, as well as in the oil after the heat treatment, even though their influence is not significant. The obtained results have shown that the cold pressed sunflower oil had a good oxidative stability at temperatures up to 63±2 °C.

Keywords: Cold-pressed sunflower oil • Oxidative stability • Specific absorbance • Storage time • Content of impurities and hulls • RSM

Study on the quality of ground, spring and river waters in South–East Serbia

Zorica S. Stojanović[1], Jaroslava V. Švarc-Gajić[1], Marika Z. Đorđević[2], Nada L. Grahovac[3], Jovica R. Vasin[3], Ana D. Đurović[1], Snežana Ž. Kravić[1]

[1] *University of Novi Sad, Faculty of Technology, Novi Sad, Serbia*
[2] *DIS Dairy Factory, Ražanj, Serbia*
[3] *Institute for Field and Vegetable Crops, Novi Sad, Serbia*

PROFESSIONAL PAPER

UDC 502.175(282)(497.11):005.1

Hem. Ind. **69** (2) 185–192 (2015)

doi: 10.2298/HEMIND131115033S

Abstract

The study deals with mineral characterisation of natural waters from South–East Serbia. The contents of aluminium, arsenic, calcium, cadmium, cobalt, chromium, cooper, iron, potassium, magnesium, manganese, sodium, nickel, lead and zinc were analysed in spring, ground and river waters by inductively coupled plasma-atomic emission spectrometry (ICP-AES) technique. The study area was in the Southern Serbia, and included slopes of Rtanj, Ozren, Bukovik, Vrdenik and Čemernik mountains, and the valley of South Morava. Obtained contents were compared with Serbian regulations on the quality of water for human use, and directive of World Health Organization (WHO) for maximum allowed concentrations of chemical substances. High contents of macro-elements, namely calcium, magnesium and potassium, were detected in several spring and ground water samples which are believed to be due to direct influence of rock minerals. Some water samples contained iron, manganese and copper in concentration up to 168.3, 8.10 and 14.9 μg dm^{-3}, respectively, but within the permissible limits. Other heavy metals were not detected in analysed samples. Based on the derived results, tested ground and spring water samples have significant potential to be used as sources for the production of bottled water, but further investigations are necessary. Additional investigations have to be focused on complete physical, chemical and microbiological assessments of water resources. Systematic hydrogeological assessment also should be performed in all seasons. In the meantime, precautionary measures should be immediately taken to protect and preserve these water resources.

Keywords: Water quality, minerals, heavy metals, water resources, water management.

Water is the most precious and essential natural resource. Out of all the water on Earth, only small fraction (about 2.5%) of it is fresh, non-saline water, suitable for human consumption [1]. Non-saline water is generally obtained from two principal natural sources: surface waters, such as lakes, rivers and streams, and ground waters, such as spring and well waters. Nowadays water resources available for human consumption are being subjected to continuous environmental stress and pollution. The pollution of water systems affects humans and the natural environment worldwide. Among wide diversity of contaminants affecting water resources, heavy metals receive particular concern considering their strong toxicity, even at low concentrations due to their accumulative effects [2]. Heavy metals exist in natural waters in colloidal, particulate and dissolved forms [3]. Although trace amount of some metals are essential for humans, any excessive exposure to heavy metals can result in adverse manifestations. The occurrence of heavy metals in ground and surface waters can be due to natural sources, such as dissolution of naturally occurring minerals containing trace elements in the soil zone. Human activities and other anthropogenic sources such as mining, fuels, smelting of ores and improper disposal of industrial wastes, significantly contribute to elevated levels of heavy metals in waters. Surface water bodies are particularly vulnerable to contamination from industrial and municipal wastewater, leaching or runoff of agrochemicals and dissolution of air-borne pollutants [4]. In ground waters greater part of the soluble constituents comes from soluble minerals in soils and sedimentary rocks and a much smaller part has its origin in the atmosphere and surface water bodies.

In Serbia there are two regulations concerning the quality of water for human use: Regulation on the hygienic acceptability of potable water [5,6], and Regulation on quality and other requirements for natural mineral water, spring water and bottled drinking water [7]. The Regulation on the hygienic acceptability of

---

Correspondence Z.S. Stojanović, Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.
E-mail: zokastojanovic@gmail.com
Paper received: 15 November, 2013
Paper accepted: 2 April, 2014

potable water defines the maximum allowed concentrations (MAC) of chemical substances in water for public water supplies. Regulation on quality and other requirements for natural mineral water, spring water and bottled drinking water defines the MAC of certain chemical parameters that can be a risk to human health, indicators of water quality and nomenclature of mineral waters. Guideline values have been determined for those chemical components that are considered to have significant potential to harm human health at concentrations above the specified limits. Guideline values should not be exceeded in public water supplies. It should also be noted that in some cases exceeding the guideline values may not always be a matter for immediate concern, but rather a trigger for a follow-up action.

Water analysis plays a decisive role in the regulations that determine water quality for specific uses, especially for drinking water quality [8]. Due to the importance of drinking water for human life, lack of water sources and the increased consumption of mineral waters, there is a need to assess the quality of water from various potential water resources. Besides this, as a part of water resources management, water protection measures need to be prioritised and implemented in order to ensure sustainability for future generations.

Serbia has significant surface water and ground water resources, sufficient to fulfil its current economic and social requirements. According to the density of water resources and the diversity of physicochemical properties of mineral waters, the territory of Serbia belongs to the one of the most resourceful areas of the European continent, but only a small quantity of these mineral waters is used for bottling [9]. In 2008 there were 30 bottling mineral water plants in Serbia [9], however, due to large number of unexamined water sources, it is believed that the potential is much higher.

The objective of this study was to evaluate the quality of spring and ground waters of the area on the slopes of Rtanj, Ozren, Bukovik, Vrdenik and Čemernik mountains and in the valley of river South Morava, in Southern Serbia. The springs of the mountains Rtanj, Ozren, Bukovik, Vrdenik and Čemernik are known for their significant amounts of water relative to Serbia and generally good quality of underground and spring waters. The examined region located at South of the Balkan Peninsula is not fully occupied by industries yet, ensuring somewhat a good quality. Water samples from river South Morava were also subject of this analysis, since some examined wells were situated close to this river. The content of aluminium, arsenic, calcium, cadmium, cobalt, chromium, cooper, iron, potassium, magnesium, manganese, sodium, nickel, lead and zinc were analysed in spring water, ground and river water. Emphasis was placed on the contents of some heavy metals such as lead, cooper and cadmium, which are very toxic, even at low concentrations due to the accumulative effects. The measurements were conducted using inductively coupled plasma-atomic emission spectrometry (ICP-AES) technique.

EXPERIMENTAL

The study area

The study area is located in the South–Eastern Serbia, and includes the slopes of Rtanj, Ozren, Bukovik, Vrdenik and Čemernik mountains and the valley of South Morava. The study region lies between the longitude 21.555 to 22.32882 and latitude 42.02009 to 43.79576. The physicogeographical position of the basin is specific due to its geographic affiliation to the mountainous Carpathian-Balkan (middle and east part) and Peripannonian (western part) regions in Serbia. The region included the territory between the valley of the South Morava down river flow in the west, Balkan mountain range in the east, the mountains Bukovik and Rtanj in the north, and the mountains Vrdenik and Čemernik in the south. Investigated area is shown in Figure 1.

Sample collection and treatment

Water samples were collected applying random and haphazard sampling from 19 different sampling sites in South-Eastern Serbia region (Figure 1). Sampling sites are described in Table 1.

Collected samples included spring, ground and river waters. River water samples were collected randomly, while ground water samples were collected by applying haphazard sampling approach [10]. Spring water samples were collected by applying a combined random and haphazard sampling approach. All samples were collected in April 2013. The river and spring water samples were collected by immersion of plastic bottles below the water surface. Groundwater samples were directly collected from wells. Before sampling, all plastic bottles were rinsed with 5% HNO_3, and then with ultrapure water. Plastic bottles were rinsed three times with the water sample before sampling. The water samples immediately upon collection were well mixed with 2 cm^3 HNO_3 (Suprapur®, Merck, Germany) per litre sample and capped tightly, in order to preserve the samples. Preserved samples were transported in ice-packed coolers to the laboratory for analysis within 24 h. Prior analyses, the samples were filtered through 0.45 µm membrane filters.

Analytical method

For element analysis in collected water samples inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used. Analysis was performed using ICP-

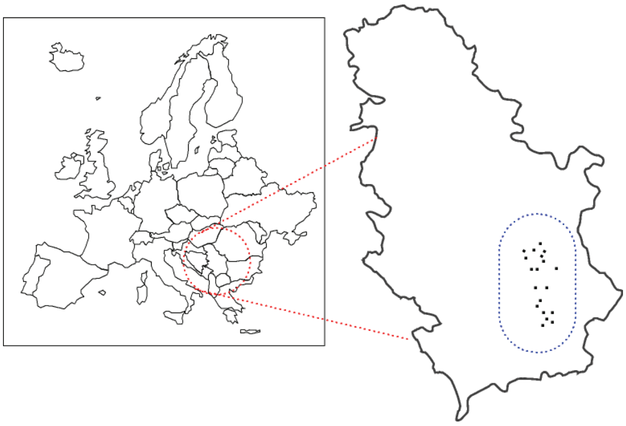


Figure 1. Map of investigated area – location of the sampling sites are marked.

Table 1. Sampling sites for different water samples

Sample number	Origin	Latitude (UTM)	Longitude (UTM)	Description
1	River water	43.561082	21.640528	8 km downstream from Aleksinac
2	River water	43.532823	21.653012	4.8 km downstream from Aleksinac
3	River water	43.527067	21.712049	Aleksinac
4	River water	43.451610	21.746339	14.5 km upstream of Aleksinac
5	River water	43.371209	21.768637	27.4 km upstream of Aleksinac
6	River water	42.834799	22.130302	Predejane
7	River water	42.699359	22.064817	Vladičin Han
8	Spring water	43.772395	21.933947	Eastern slope of Rtanj
9	Spring water	43.795756	21.760225	Northwestern slope of Rtanj, Krivi vir
10	Spring water	43.625265	21.853867	Northeastern slope of Ozren, Gradašnica
11	Spring water	43.551985	21.812711	Southern slope of Ozren, Lipovac
12	Spring water	43.551658	21.810265	Southern slope of Ozren, Lipovac
13	Spring water	43.72211	21.555004	Northwestern slope of Bukovik, Šetka
14	Ground water	42.767962	22.087643	Džep
15	Ground water	42.691647	22.175345	Surdulica
16	Ground water	43.293247	22.006881	Niška banja
17	Ground water	43.560989	21.833879	Southern slope of Ozren, Lipovac
18	Ground water	42.760121	22.178307	Southwestern slope of Čemernik, Mačkatica
19	Ground water	42.742773	22.328821	Vlasina Rid

-OES, Varian Vista Pro-axial. Concentrations of heavy metals and trace elements were determined by US EPA method 200.7 [11]. Samples were analysed directly by pneumatic nebulization without acid digestion. Stock standards were purchased from J.T. Baker, BAKER Instra-Analysed Reagent Grade (J.T. Baker® 5724 Calcium Plasma Standard, J.T. Baker® 5741 Potassium Plasma Standard, J.T. Baker® 5734 Magnesium Plasma Standard, J.T. Baker® 5746 Sodium, Baker Instra-Analyzed® Reagent Grade and J.T. Baker® 6031-01 Trace Metal Standard I Al, As, Cd, Co, Cr,Cu, Fe, Mn, Ni, Pb, Zn).

RESULTS AND DISCUSSION

The results on elements contents in the analysed water samples are given in the Table 2. Comparison of Regulations in Serbia with directive of World Health Organization [12] for maximum allowed concentrations of chemical substances is also shown in Table 2.

The obtained results indicated that the concentrations of almost all of the analysed elements and heavy metals in water samples were considerably below the maximum allowed concentrations (MAC) stipulated in Official Gazette of the Republic of Serbia and WHO (Figure 2).

The concentrations of calcium in analysed samples were in the range from 31.4 to 180 mg dm^{-3}. All samples met the Regulations for calcium for public water use, but not for natural mineral water, spring water and bottled drinking water. Due to high calcium content, only samples 12, 13, 14 and 19 could be clas-

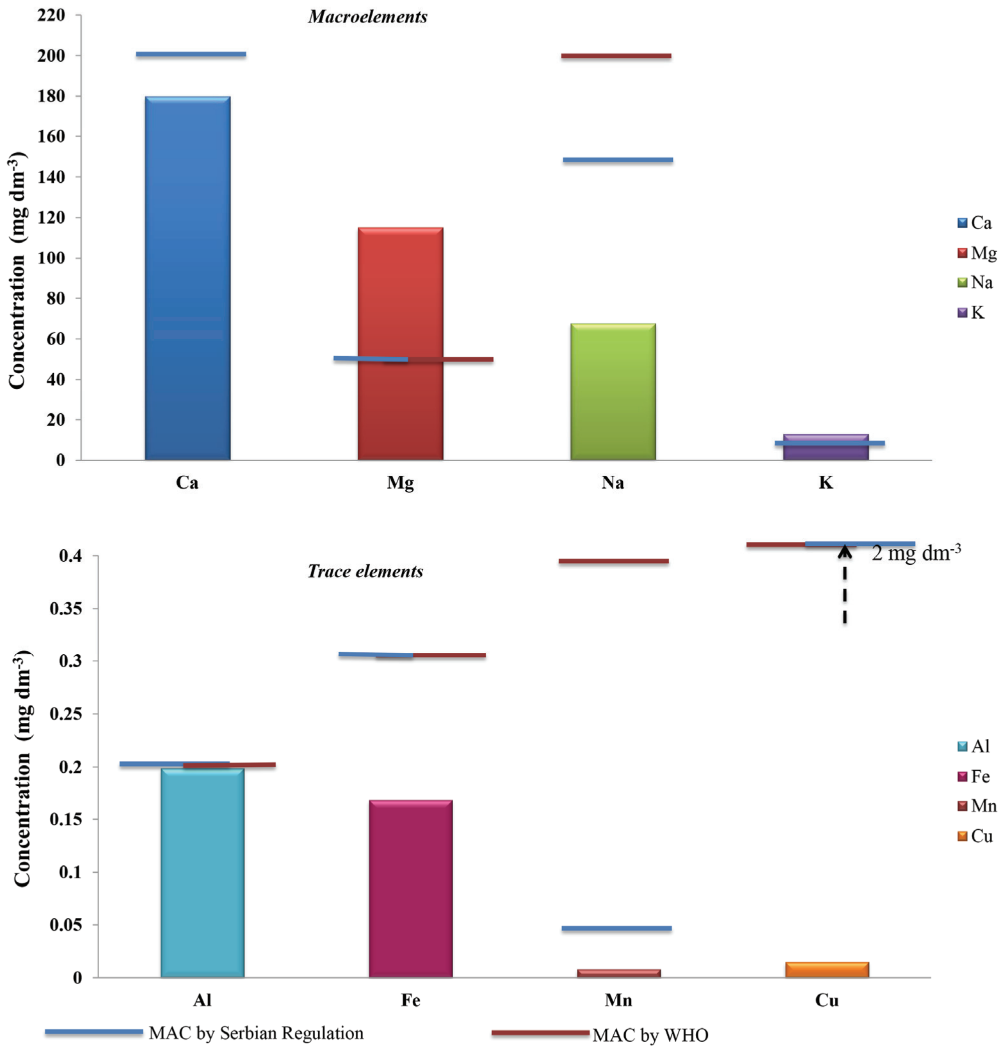


Figure 2. Maximum concentrations of micro, macro and trace elements determined in water samples compared with maximum allowed concentrations.

Table 2. Concentrations of trace and major elements in the analysed water samples (mg dm^{-3}); nd – not detected

Sample	Element														
	Ca	Mg	Na	K	Al	As	Fe	Mn	Cd	Co	Cr	Cu	Ni	Zn	Pb
1	85.5	13.28	10.99	2.79	nd[a]	nd	0.013	0.002	nd	nd	nd	nd	nd	nd	nd
2	93.7	23.45	22.71	4.07	nd	nd	nd	0.003	nd	nd	nd	nd	nd	nd	nd
3	76.9	17.06	31.24	6.18	nd	nd	0.026	0.005	nd	nd	nd	nd	nd	nd	nd
4	75.6	12.24	13.57	3.72	0.182	nd	0.004	nd	nd	nd	nd	nd	nd	nd	nd
5	31.4	7.29	18.68	4.42	0.199	nd	0.142	0.006	nd	nd	nd	nd	nd	nd	nd
6	91.1	25.80	25.47	3.13	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	35.8	12.18	13.27	7.73	nd	nd	0.168	0.008	nd	nd	nd	nd	nd	nd	nd
8	136.0	9.16	4.33	1.41	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
9	112.5	19.60	5.64	2.56	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	113.6	19.75	5.88	2.55	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11	94.6	8.20	3.54	0.69	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	150.6	29.31	34.30	1.96	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	158.3	73.69	39.58	0.73	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
14	174.9	115.20	67.77	2.18	nd	nd	0.084	0.005	nd	nd	nd	nd	nd	nd	nd

Table 2. Continued

Sample	Element														
	Ca	Mg	Na	K	Al	As	Fe	Mn	Cd	Co	Cr	Cu	Ni	Zn	Pb
15	125.1	47.27	54.46	0.39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	124.3	37.89	64.17	0.91	nd	nd	nd	nd	nd	nd	nd	0.015	nd	nd	nd
17	148.1	49.53	65.61	2.21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18	103.9	51.75	65.41	12.88	nd	nd	0.011	0.003	nd	nd	nd	nd	nd	nd	nd
19	180.0	90.12	38.99	0.75	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MAC[a]	200	50	150	12	0.2	0.01	0.3	0.05	0.003	–	0.05	0.02	2	3	0.01
MAC[b]	150[c]	50[c]	200[c]	–	0.2	0.01	0.2	0.05	0.003	–	0.05	0.02	2	–	0.01
WHO[d]	–	50	200	–	0.2	0.01	0.3	0.4	0.003	–	0.05	0.07	2	–	0.01

[a] Maximum allowed concentrations, Guideline value given by the Regulation on the hygienic acceptability of potable water [5,6], MAC of chemical substances in water for public water supply; [b] maximum allowed concentrations, Guideline value given by the Regulation on quality and other requirements for natural mineral water, spring water, and bottled water [7]; [c] lower limit of nomenclature; [d] Guideline values of WHO [12]

sified as mineral waters in accordance with Serbian Regulation on quality and other requirements for natural mineral water, spring water and bottled drinking water. Magnesium content in examined water samples varied from 7.3 to 115.2 mg dm^{-3}. Samples 13, 14, 18 and 19 had content higher of those permitted by Serbian Regulation and by the WHO, however those samples met requirements for natural mineral water, spring water and bottled drinking waters. Drinking water with high levels of calcium and magnesium could provide important health benefits. Magnesium is very helpful in the prevention of some diseases, including hypertension, fluctuation of blood sugar levels, heart arrhythmia, endocrinologic diseases and nervous system diseases. Calcium may be helpful in osteoporosis for many people whose dietary intake is deficient in this nutrient [12]. A review of published data [13] showed that bioavailability of the calcium from waters is comparable to bioavailability of calcium from milk, when studied under similar conditions. Heaney concluded that calcium in high-calcium mineral waters is highly absorbable, noting that high-calcium water functions much like a supplement [13]. Also, waterborne magnesium is more bioavailable than magnesium obtained from foods and water may be a significant source of magnesium [14]. Although studies show that calcium and magnesium are useful minerals in water, there are no guidelines on minimum concentrations of these minerals in drinking water. Contents of calcium and magnesium in water are associated with water hardness. Hard water is not a health risk, but is a nuisance because of mineral build up in plumbings and poor soap and/or detergent performance, if such water is used in household or in industry.

Most water supplies contain less than 20 mg of sodium per litre, but in some countries levels can exceed 250 mg dm^{-3} [15]. Saline intrusion, mineral deposits, seawater spray, sewage effluents, and salt used in road de-icing can all contribute significant quantities of sodium in water. The relationship between elevated sodium intake and hypertension has been the subject of considerable scientific controversy, but on the basis of existing data, no firm conclusions can be drawn concerning the possible association between sodium in drinking water and the occurrence of hypertension [15]. No health-based guideline value is therefore proposed. However, sodium may affect the taste of a drinking-water at levels above 200 mg dm^{-3} [15]. All analysed samples had satisfactory content of sodium (3.5–67.8 mg dm^{-3}).

Potassium is an essential element in humans. It occurs widely in the environment, including all natural waters, in low concentrations. Adverse health effects due to potassium consumption from drinking-water are unlikely to occur in healthy individuals, because it is rapidly excreted in the absence of pre-existing kidney damage. Consequently it is not considered necessary to establish a health-based guideline value for potassium in drinking-water. In analysed samples potassium content was in the range of 0.4–7.7 mg dm^{-3}, and the exception was the sample 18, where its content was 12.88 mg dm^{-3}. Only this sample had a higher content of potassium than allowed by Serbian regulations for chemical substances in water for public water supply.

Aluminium is one of the most abundant elements found in the Earth's crust. Toxicity of aluminium is associated with continuous low level exposure, which can eventually lead to serious health effects such as Alzheimer's and Parkinson's disease, senility and presenile dementia [2]. Results obtained showed that aluminium was detected in two samples at levels below MAC (0.182–0.199 mg dm^{-3}).

Arsenic is found widely in the Earth's crust as sulphides, or metal arsenides or arsenates. It is usually present in natural waters at concentrations less than 1–2 mg dm^{-3} [12], but in ground water, its concentration can be significantly elevated. Many studies have shown that long-term exposure to arsenic in drinking-water is related to increase risks of cancer in the skin, lungs, and kidneys [12]. In analysed samples arsenic was not detected.

Iron is found in natural fresh waters, especially in ground waters. Ground water containing soluble iron may remain clean when pumped out, but exposure to air may cause precipitation of iron due to oxidation, which causes brownish-red colour. Iron affects target organs such as liver, cardiovascular system and kidneys [16]. All water samples were with iron content below the allowed limits (4.4–168.3 μg dm^{-3}).

Manganese concentration levels ranged from 2.2 to 8.1 μg dm^{-3} and met all the set guidelines. At higher concentration levels (higher than 0.1 mg dm^{-3}), manganese causes an undesirable taste in drinking-water, and precipitates due to oxidation of Mn(II) to Mn(IV) resulting in stain household utensils and clothes [17]. The presence of manganese in drinking-water may also lead to the accumulation of deposits in the distribution systems [12].

Cadmium has carcinogenic properties as well as long biological half-life, leading to chronic effects as a result of accumulation in the liver [12,18]. It can also cause kidney damage, as well as produce acute health effects resulting from overexposure to high concentrations [18]. Cadmium was not detected in any of the analysed samples.

Cobalt has both beneficial and harmful effects on human health. It is beneficial for humans as a part of vitamin B12, which is essential to human health. At elevated levels cobalt provokes harmful health effects. It can cause pulmonary syndrome, skin syndrome, allergy, gastrointestinal irritations, nausea, cardiomyopathy, haematological disorders [19]. Fortunately, cobalt is rarely detected in drinking-water. According to WHO, the concentration of cobalt in drinking-water is low and ranges from 0.1 to 5 μg dm^{-3} [12]. In analysed water samples cobalt was not detected.

Chromium is present in small quantities in the environment. The toxicity of chromium depends on its physicochemical form; hexavalent salts are considered the most dangerous [20]. Prolonged consumption of water containing elevated concentrations of chromium, can damage liver, kidney and nervous tissue [21]. Chromium was not detected in any of the analysed samples.

Copper as many other metals can exist in aquatic environment in three forms, namely soluble, colloidal and particulate. At lower doses it is essential element for organisms. Excess of copper in human body is toxic and causes hypertension and produces pathological changes in brain tissues [12]. Of all analysed water samples, only sample 16 contained copper (0.015 mg dm^{-3}), but in concentration much lower than permitted.

Long-term exposure to elevated concentrations of nickel causes weight loss, heart and liver damage, and dermatitis [21]. In all analysed samples nickel was not detected.

Zinc is an essential trace mineral that is found in water and food in the form of salts or organic complexes. Although concentrations of zinc in surface and ground waters do not exceed 10 or 50 μg dm^{-3}, concentration of zinc in tap water can be much higher, due to the dissolution of the element from pipes and valves materials [21]. Zinc was not detected in any of the analysed samples.

Lead occurs naturally in the environment. However, the most significant concentrations of this element found in the environment are the result of the human activities. Lead is toxic and it accumulates in kidneys and skeleton. Over centuries lead became recognised as a cumulative general metabolic poison. Lead contamination of the ground waters may result from industrial effluents, old plumbing household sewages, agricultural run-off containing phosphatic fertilizers, and human and animal excreta [16]. Lead was not detected in any of the analysed samples.

Previous similar investigations focusing on heavy metal determinations in spring and river water samples in Serbia showed the presence of cadmium, lead, zinc and nickel [22,23]. On the basis of the obtained results and by comparison with the world average contents of trace elements for unpolluted rivers, contents of heavy metals in analyzed water samples from the river South Morava were very low, classifying those river sites as unpolluted at the time of sampling. Additional experiments are in progress to provide reliable statistic results in order to examine if these alluvial deposits may be used as good sources of water supply to many communities close to this river.

With industry development the heavy metal pollution became an issue of global proportions. The fact that heavy metals did not exceed permitted limits in analysed water samples favours the quality of water from investigated area. On the other hand, some analysed water samples cannot be used for public water supply, due to calcium and magnesium level higher than permitted. These waters, however, can be used as bottled drinking waters according to Serbian regulations [7]. For such waters elevated level of specific mineral should be clearly labelled. As a result, on Serbian markets natural waters rich in different minerals (magnesium and potassium) can be found.

CONCLUSIONS

According to conducted study focusing on quality control of natural spring, river and ground waters in South-East Serbia, it can be concluded that majority of samples were free of heavy metals. The exceptions were iron, manganese, and copper found in some samples, but their contents were within allowed limits. As for major elements, including calcium, magnesium and potassium, some samples had higher contents of those specified in the guidelines. Elevated levels of calcium were detected in two samples of spring waters and two samples of ground waters, whereas increased potassium concentration was observed in one sample of ground water. These samples, however, fulfilled the requirements of WHO in respect to specified maximum calcium and potassium levels. Levels of magnesium, higher of those specified by Serbian regulations and by WHO, were found in three ground water samples and one spring water sample. Data on the quality of unused water sources are sparse, especially for the studied region, and on the basis of obtained results a great potential for new water resources are recognized, especially for the production of bottled water. These preliminary results highlight the need for further investigations that should be focused on microbiological quality as well as organic chemical pollutants which may be present in water sources. In addition, hydrogeological assessment of abundance and assessments at periodic intervals are necessary to verify the effectiveness of management decisions of ground and spring water resources exploitation. In the meantime, to protect and preserve these water resources certain measures should be immediately taken. The preservation of water resources should be performed in economically, socially, and environmentally sustainable manner.

Acknowledgment

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia through national project number TR 31014.

REFERENCES

[1] H.K. Okoro, A. Adeyinka, O.E. Jondiko, B.J. Ximba, S.J. Kakalanga, Assessment of heavy metals contamination in groundwater: A case study of central industrial district in Ilorin, Kwara State, Nigeria, Int. J. Phys. Sci. **7** (2012) 5078–5088.

[2] M.A. Momodu, C.A. Anyakora, Heavy metal contamination of ground water: The surulere case study, Res. J. Environ. Earth Sci. **2** (2010) 39–43.

[3] M. Zabochnicka-Swiatek, O. Gyliene, K. Cederkvist, P.E. Holm, in: M. Ersoz, L. Barrott (Eds.), Best practice guide on metals removal from drinking water by treatment, 2012, IWA Publishing, London, 2012, pp. 61–70.

[4] F. Malaguerra, H-J. Albrechtsen, P.J. Binning, Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques, J. Hydrol. **476** (2013) 321–331.

[5] Regulation on the hygienic acceptability of potable water, Official Gazette of FRY, No. 42/98, 1998.

[6] Regulation on the hygienic acceptability of potable water, Official Gazette of FRY, No. 44/99, 1999.

[7] Regulation of quality and other requirements for natural mineral water, spring water and bottled drinking water, Official Gazette of Serbia and Montenegro, No. 53/05, 2005.

[8] M. Ristić, I. Popović, V. Pocajt, D. Antanasijević, A. Perić-Grujić, Concentration of selected trace elements in mineral and spring bottled waters on the Serbian market, Food Addit. Contam., B **4** (2011) 6–14.

[9] T. Petrović, M. Zlokolica-Mandić, N. Veljković, D. Vidojević, Hydrogeological conditions for the forming and quality of mineral waters in Serbia, J. Geochem. Explor. **107** (2010) 373–381.

[10] J. Švarc-Gajić, Merna nesigurnost, Tehnološki fakultet, Novi Sad, 2013.

[11] Method 200.7: Determination of trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry, Revision 4.4, EMMC Version, Method for the determination of metals in environmental samples, Supplement I, EPA/600/R-94-111, US EPA, 1994.

[12] World Health Organization, Guidelines for drinking-water quality, 3rd ed., incorporating the first and second addenda, Vol. 1, Recommendations, Geneva, 2008.

[13] R.P. Heaney, Absorbability and utility of calcium in mineral waters. Am. J. Clin. Nutr. **84** (2006) 371–374.

[14] J. Durlach, M. Bara, A. Guiet-Bara, Magnesium level in drinking water and cardiovascular risk factor: a hypothesis, Magnesium **4** (1985) 5–15.

[15] World Health Organization, Sodium in Drinking-water Background document for development of WHO, Guidelines for drinking-water quality, 2nd ed., Vol. 2, Health criteria and other supporting information, Geneva, 1996.

[16] A.A. Jameel, J. Sirajudeen, A. Abdul vahith, Studies on heavy metal pollution of ground water sources between Tamilnadu and Pondicherry, India. Adv. Appl. Sci. Res. **3** (2012) 424–429.

[17] Z. Teng, J.Y. Huang, K. Fujita, S. Takizawa, Manganese removal by hollow fiber micro-filter. Membrane separation for drinking water, Desalination **139** (2001) 411–418.

[18] J. Švarc-Gajić, General Toxicology, Nova Publishers, New York, 2009.

[19] G.M. Farías, S. Moyano, J.A. Gásquez, R. Wuilloud, L.D. Martínez, Determination of cobalt by ICP-AES in drinking-water samples using a knotted reactor with flow-injection on-line, J. Argent. Chem. Soc. **90** (2002) 77–86.

[20] E. Derwich, Z. Benziane, L. Benaabidate, D. Belghyti, Impact of urban wastewaters on groundwater quality in the watershed of Midde Sebou (Morocco), J. Black Sea/Mediterr. Environ. **15** (2009) 157–164.

[21] D.Z. Antanasijević, N.A. Lukić, V.V. Pocajt, A.A. Perić-Grujić, M.Đ. Ristić, Analysis of selected elements in water in the drinking water preparation plants in Belgrade, Serbia, Hem. Ind. **65** (2011) 187–196.

[22] M.B. Rajković, J.D. Sredović, M.B. Račović, M.D. Stojanović, Analysis of quality mineral water of Serbia: Region Arandjelovac, J. Water Resour. Prot. **4** (2012) 783–794.

[23] S. Stanković, D. Čičkarić, J. Marković, Determination of Pb and Cd in water by potentiometric stripping analysis (PSA), Desalination **213** (2007) 282–287.

IZVOD

ISPITIVANJE KVALITETA PODZEMNIH, IZVORSKIH I REČNIH VODA SA PODRUČJA JUGOISTOČNE SRBIJE

Zorica S. Stojanović[1], Jaroslava V. Švarc-Gajić[1], Marika Z. Đorđević[2], Nada L. Grahovac[3], Jovica R. Vasin[3], Ana D. Đurović[1], Snežana Ž. Kravić[1]

[1]*Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, Srbija*
[2]*Mlekara DIS, Ražanj, Srbija*
[3]*Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija*

(Stručni rad)

Ovaj rad predstavlja analizu kvaliteta prirodnih voda iz jugoistočne Srbije. Sadržaj arsena, aluminijuma, kalcijuma, kadmijuma, kobalta, hroma, bakra, gvožđa, kalijuma, magnezijuma, mangana, natrijuma, nikla, olova i cinka je određivan u izvorskim i podzemnim vodama, kao i u rečnoj vodi. Analiza navedenih elemenata je izvedena ICP-AES tehnikom. Analizirani su uzorci voda iz oblasti jugoistočne Srbije, uključujući područja planine Rtnja, Ozrena, Bukovika, Vrdenika i Čemernika, kao i područje doline reke Južne Morave. Određeni sadržaji analiziranih elemenata su poređeni sa važećim pravilnicima Republike Srbije i preporukama Svetske zdravstvene organizacije (SZO). Visoki sadržaj makro-elemenata, odnosno kalcijuma, magnezijuma i kalijuma uočeni su u izvorskim i nekoliko uzoraka podzemnih voda, što može biti posledica direktnog uticaja magmatskih stena. Pojedini uzorci voda su sadržali nešto više sadržaje gvožđa, mangana i bakra u koncentraciji do 168,3, 8,10 i 14,9 μg dm^{-3}, redom, ali u granicama propisanim Pravilnikom i u skladu da preporukama SZO. Ostali teški metali nisu detektovani u ispitivanim uzorcima. Na osnovu dobijenih rezultata se može zaključiti da testirani uzorci podzemnih i izvorskih voda imaju značajan potencijal u smislu proizvodnje flaširane vode, uz neophodna dodatna istraživanja. Dodatna istraživanja bi trebalo usmeriti na kompletnu hemijsku analizu i ispitivanje fizičkih i mikrobioloških karakteristika uzoraka voda. Pored toga, neophodno je sprovesti sistematsko hidrogeološko ispitivanje izdašnosti podzemnih i izvorskih voda tokom svih godišnjih doba. U međuvremenu treba preduzeti mere predostrožnosti kako bi se ovi vodeni resursi zaštitili i očuvali.

Ključne reči: Kvalitet vode • Minerali • Teški metali • Vodeni resursi • Upravljanje vodenim resursima

Sposobnost upravljanja vlagom glatkih pletenina izrađenih od prirodnih i regenerisanih celuloznih vlakana

Milada S. Novaković[1,2], **Lana S. Putić**[2], **Matejka Bizjak**[3], **Snežana B. Stanković**[2]

[1]*Visoka tehnička škola strukovnih studija, Zrenjanin, Srbija*
[2]*Univerzitet u Beogradu, Tehnološko–metalurški fakultet, Begrad, Srbija*
[3]*Univesity of Ljubljana, Faculty of natural sciences and engineering, Ljubljana, Slovenia*

NAUČNI RAD

UDK 677.075.017

Hem. Ind. **69** (2) 193–200 (2015)

doi: 10.2298/HEMIND140201034N

Izvod

Cilj ovog rada je bio karakterizacija glatkih pletenina u pogledu sposobnosti upravljanja vlagom (propuštanja vodene pare i znoja) uzimajući u obzir faktore na mikroskopskom (vlakna), mezoskopskom (pređa) i makroskopskom (pletenina) nivou. U tu svrhu su, polazeći od pređa izrađenih od prirodnih i regenerisanih celuloznih vlakana, u kontrolisanim uslovima proizvedene glatke DL pletenine koje su podvrgnute ispitivanju propustljivosti vodene pare i tečnosti. Dobijeni rezultati ukazali su na kompleksan uticaj hijerarhijske strukture tekstilnog materijala na njegovu sposobnost upravljanja tečnostima. Pokazalo se da pored distribucije pora u pleteninama, koja je u okviru eksperimentalnog materijala bila uslovljena deformacionim svojstvima vlakana i primenjenim tehnikama predenja, propustljivost vodene pare može biti modifikovana geometrijom površine pređe, dok morfologija vlakana može da modifikuje propustljivost tečnosti.

Ključne reči: pletenina, upravljanje vlagom, propustljivost vodene pare, kapilarno kvašenje, poroznost, maljavost pređe.

Čovekov organizam se može posmatrati kao komplikovan termodinamički sistem u kome se metaboličkim procesima energija kontinuirano proizvodi, i koja se kontinuirano oslobađa u obliku toplote ili latentnih gubitaka toplote koji prate isparavanje znoja. Vršeći svoju osnovnu funkciju – zaštitu od ekstremnih spoljnih uticaja, odeća je u stalnom kontaktu sa čovekovim telom formirajući pri tome mikroklimu ili mikrookruženje koje je od izuzetne važnosti za čovekovu percepciju toplotnog komfora. Drugim rečima, odeća predstavlja u određenoj meri barijeru slobodnoj razmeni energije i mase čoveka sa njegovim okruženjem. Sa aspekta toplotnog komfora od odeće se očekuje da aktivno podrži čovekov termoregulacioni sistem u održavanju adekvatne temperature čovečijeg tela, uz obezbeđivanje što je moguće manje vlage u mikroklimi. Osim dizajna i veličine odevnog predmeta, suštinski značaj u pogledu toplotnog komfora ima sposobnost tekstilnog materijala da prenosi toplotu i propušta vazduh i vodenu paru (znoj). Međutim, vrlo često se sposobnost tekstilnog materijala da prenosi vodu (znoj u tečnom stanju) uzima kao ključni parametar toplotnog komfora. To su situacije kada se odevni predmet koristi u uslovima većih fizičkih aktivnosti, ili u uslovima čestih promena fizičke aktivnosti i klimatskih uslova okruženja kada dolazi do vremenskog zaostajanja u termoregulaciji ljudskog organizma. U tzv. normalnim uslovima, koji podrazumevaju uobičajeni nivo fizičke aktivnosti i neekstremne klimatske uslove, telo oslobodi oko 60 ml vodene pare u toku jednog sata. Ovakvo latentno ili neprimetno znojenje ili isparavanje vlage u obliku pare čini 15% ukupno oslobođene toplote kroz kožu. Jača fizička aktivnost izaziva porast energije metabolizma pri čemu znojne žlezde luče 450 ml/h znoja [1]. Ukoliko tečni znoj ne bude dovoljno brzo odveden sa površine kože, narušava se osećaj komfora. Oslobađanje toplote isparavanjem tečnog znoja je izuzetno važno i u uslovima visokih ambijentalnih temperatura kada su kondukcija, konvekcija i radijacija smanjeni usled niskog temperaturnog gradijenta. U slučaju kada je prisutan temperaturni gradijent suprotnog smera između kože i okruženja, isparavanje znoja ostaje jedini način da se telo oslobodi viška toplote. S obzirom da je latentna toplota isparavanja vode 2500 kJ/kg, čak i mala isparena količina znoja značajno doprinosi ukupnom toku toplote.

Istraživanja su pokazala da se, u zavisnosti od agregatnog stanja, znoj različitim fizičkim mehanizmima prenosi kroz tekstilne materijale. Znoj u obliku pare se prenosi molekulskom difuzijom kroz vlakna, površinskom difuzijom adsorbovanih molekula na površini vlakana i molekulskom difuzijom kroz pore u materijalu. Pri tome može da dođe do apsorcpije vlage što zavisi od hemijske prirode vlakana. Znoj u obliku tečnosti se prenosi mehanizmom adsorpcije po površini vlakana i pređe i mehanizmom kapilarnog kvašenja, pri čemu su ovi mehanizmi uslovljeni hidrofilnošću površine vla-

---

Prepiska: S. Stanković, Tehnološko–metalurški fakultet, Karnegijeva 4, 11120 Beograd, Srbija.
E-pošta: stankovic@tmf.bg.ac.rs
Rad primljen: 1. februar, 2014
Rad prihvaćen: 2. april, 2014

kana. Tečnost može da se zadrži u međuprostorima između vlakana ili da se apsorbuje vlaknima.

Pored sposobnosti da odvede vlagu sa površine kože, od tekstilnog materijala se generalno očekuje da se sam brzo oslobodi vlage, odnosno da se brzo suši. U suprotnom, vlaga koje se zadržava u materijalu izaziva osećaj hladnoće i narušava osećaj komfora. Sposobnost tekstilnih materijala da prenose paru i tečnost je u međunarodnoj literaturi poznata pod zajedničkim imenom svojstva upravljanja vlagom (eng. *moisture management properties*). Bilo da se radi o prenosu znoja u obliku pare ili tečnosti, reč je o komplikovanim procesima koji su uslovljeni nizom faktora kao što su sirovinski sastav (hidrofilna ili hidrofobna priroda vlakana), poroznost i debljina materijala [2]. Polazeći od poznate hidrofilne prirode celuloznih vlakana, ali imajući u vidu strukturne parametre tekstilnog materijala koji definišu njegovo ponašanje u pogledu sposobnosti prenosa vlage, u okviru ovog rada su ispitivana svojstva upravljanja tečnostima glatkih pletenina izrađenih od prirodnih i regenerisanih celuloznih vlakana. Iako je relativno veliki broj naučnih radova posvećen ispitivanjima ovih svojstava tekstilnih materijala [3–15], u okviru ovog rada učinjen je pokušaj analize kompleksnog uticaja strukturnih parametara pletenina imajući u vidu njihovu tipičnu hijerarhijsku strukturu koju čine tri nivoa: mikroskopski (vlakna), mezoskopski (pređa) i makroskopski (pletenina).

EKSPERIMENTALNI DEO

Materijal

U okviru ovog istraživanja korišćene su štapel jednožične pređe od konoplje (Linificio Canapificio Nazionale, Italija), pamuka (PVK, Vranje) i viskoze (Viskoza, Loznica) iste nominalne finoće 50 tex sa upredanjem u Z smeru. Nominalne i faktičke karakteristike jednožičnih ispitivanih pređa date su u tabeli 1. Provera faktičkih svojstava ispitivanih pređa sprovedena je prema standardima SRPS ISO 2060 [16] i SRPS ISO 2061 [17].

Prethodnim dubliranjem jednožičnih štapel pređa omogućeno je dobijanje homogene pamučne, viskozne i pređe od konoplje i dubliranih nehomogenih konoplja/pamuk i konoplja/viskoza pređa. Na kružnoj pletaćoj mašini finoće NoE 20 od ovih pređa su izrađene glatke desno-leve (DL) pletenine čije su konstrukcione karakteristike date u tabeli 2.

Debljina pletenina je određena prema standardu SRPS EN ISO 5084 [18]. Ostali strukturni parametri proizvedenih pletenina određeni su prema uobičajenoj proceduri opisanoj u literaturi [19]. Sva ispitivanja vršena su u kondicionim uslovima (20±2 °C, Rh 65±2%). DL pletenine su podvrgnute ispitivanjima koja se odnose na sposobnost propuštanja vodene pare i tečnosti.

Metode ispitivanja

Pređe su podvrgnute ispitivanju geometrije površine, dok su svojstva upravljanja vlagom glatkih DL pletenina ocenjena upotrebom dve metode. Propustljivost vodene pare je ocenjena parametrima – otpornost prema propuštanju vodene pare i propustljivost vodene pare. Sposobnost propuštanja vode ispitivana je nešto modifikovanim testom distribucije vode koji je razvijen od strane kompanije Polartec® LLC (Lawrence, MA, USA), a u stručnoj literaturi je poznat pod nazivom Malden Mills test, kako glasi stari naziv kompanije.

Geometrija površine pređe ocenjena je parametrom – maljavost pređe. Za ispitivanje maljavosti pređe korišćen je instrument Shirley Hairiness Monitor SDL 103. Instrument sačinjavaju merna i računska jedinica. Mernu jedinicu čine izvor svetlosti i fotodioda, tako da prilikom prolaženja pređe kroz mernu glavu (55–100

Tabela 1. Karakteristike jednožičnih pređa
Table 1. Characteristics of the single yarns

Vlakno	Finoća, tex		Upredanje, m^{-1}		Koeficijent upredanja, $tex^{1/2}$ cm^{-1}	
	Nominalno	Faktičko	Nominalno	Faktičko	Nominalno	Faktičko
Konoplja	50	47,8	400	370	28,3	25,6
Pamuk	50	48,9	490	475	34,6	33,2
Viskoza	50	44,4	400	340	28,3	22,6

Tabela 2. Konstrukcione karakteristike DL pletenina
Table 2. Construction characteristics of the plain knitted fabrics

Sirovinski sastav	Gustina petlji, cm^{-1}		Površinska gustina cm^{-2}	Dužina petlje mm	Debljina mm	Površinska masa $g\ m^{-2}$
	D_h	D_v				
Konoplja+konoplja	5,5	13,7	75,4	5,0	0,916	360,4
Pamuk+pamuk	6,0	12,0	72,0	5,4	1,163	387,3
Viskoza+viskoza	6,0	13,0	78,0	5,3	1,048	367,1
Konoplja+pamuk	5,5	13,0	71,5	5,3	1,047	366,4
Konoplja+viskoza	5,5	14,0	77,0	5,3	0,957	376,3

m/min) prisutna štrčeća vlakna prekidaju za trenutak svetlosni fluks. Pojačani signal sa fotodiode, kao funkcija smanjenja svetlosti koja pada na fotoćeliju, prenosi se u računsku jedinicu koja obezbeđuje digitalni zapis. Korišćena varijanta instrumenta registruje malje duže od 3 mm sa odsečka pređe od 70° tela pređe, uz mogućnost podešavanja vremena merenja u određenom intervalu i kontinualno. Za potrebe ovog istraživanja izabran je vremenski interval od 5 s, budući da se pokazalo da se upotrebom tog intervala mogu dobiti reprezentativni rezultati. Za svaku pređu izvrešno je po 30 merenja maljavosti (broja malja registrovanih u toku 5 s). Srednje vrednosti ovih rezultata iskazane su kao broj malja po 1 m pređe.

Ispitivanja propustljivosti vodene pare vršena su na Permetest-u (Sensora Instruments, Češka) po proceduri nešto izmenjenoj u odnosu na standard ISO 11092 (Textiles – Physiological effects-Measurement of the thermal and water-vapour resistance). Izmene se odnose na primenu izotermičkih uslova merenja, koji podrazumevaju održavanje temperature u intervalu 20–22 °C što odgovara ambijentalnoj temperaturi. Pored toga, umesto relativne vlažnosti paralelnog toka vazduha od 40%, ispitivanja su vršena pri relativnoj vlažnosti od 60–65% (ambijentalna vlažnost) [20]. Permetest predstavlja mali „model kože“. Merna glava ovog uređaja, sa površinom od poroznog metalnog sloja, pokrivena je polupropustljivom teflonskom membranom koja je propustljiva za vodenu paru ali ne i za tečnost. Toplotni fluks, nastao kao posledica isparavanja vode sa porozne površine, meri se specijalnim senzorom koji čini integralni deo poroznog sloja. Posredstvom pojačivača signal se šalje digitalnom indikatoru električnog napona koji registruje napon u mV. U sledećem koraku polupropustljiva površina merne glave se pokriva uzorkom pletenine i po uspostavljanju stabilnog signala registruje se nivo napona koji karakteriše gubitke toplote u prisustvu pletenine. Opisana procedura je ponovljena tri puta za svaku ispitivanu pleteninu. Na osnovu registrovanih vrednosti napona, odnosno toplotnog fluksa za ispitivane pletenine, i shodno standardu ISO 11092 [21] izračunate su vrednosti otpornosti pletenina prema propuštanju vodene pare (R_{et}) (m^2 Pa W^{-1}) prema relaciji (1):

$$\begin{aligned} R_{et} &= \left(p_{vz}-p_{v}\right)\left(\frac{1}{S_sU_o}-\frac{1}{S_sU_s}\right)= \\ &= C_{ref}\left(100-\phi\right)\left(\frac{1}{q_s}-\frac{1}{q_0}\right) \end{aligned} \qquad (1)$$

gde je p_{vz} (Pa) – parcijalni pritisak vodene pare u slučaju zasićenog vazduha na datoj temperaturi, p_{v} (Pa) – aktuelni parcijalni pritisak vodene pare, U_0 i U_{s} – registrovani električni napon u mV za mernu glavu bez pletenine i u slučaju fiksiranja uzorka preko merne glave, ϕ (%) – relativna vlažnost vazduha, q_{s} i q_0 – srednja vrednost toplotnog fluksa u W m^{-2} u prisustvu uzorka pletenine i bez njega i S_{s} (W m^{-2} mV^{-1}) – osetljivost senzora (koji meri toplotni fluks). Konstanta C_{ref} se određuje kalibracijom uz upotrebu referentnog uzorka poznate otpornosti isparavanju (R_{et} = 2,36 m^2 Pa W^{-1}).

Relativna propustljivost vodene pare, P_{wv} (%), DL pletenina je izračunata kao količnik registrovanih vrednosti električnog napona u prisustvu uzorka pletenine i bez njega.

Malden Mills test distribucije vode koji je poslužio za ocenu sposobnosti pletenina da propuštaju vodu ne podrazumeva upotrebu posebnog uređaja. Prema proceduri, uzorak pletenine se postavlja horizontalno između dva filter papira tako okrenutih da njihova apsorbujuća strana bude u kontaktu sa uzorkom. Na površinu pletenine koja je okrenuta na gore nanosi se određena zapremina vode (2 ml). Posle perioda stabilizacije (2 min) preko gornjeg filter papira postavlja se opterećenje (500 g), i posle jednog minuta meri se masa gornjeg i donjeg filter papira. Voda apsorbovana u donjem (W_{d}) i gornjem (W_{g}) filter papiru omogućuje da se odgovarajućim proračunom dođe do podataka koji definišu distribuciju vode na desnoj (licu) i levoj (naličju) strani pletenine. Kada se na levu stranu pletenine nanese određena zapremina vode, količina vode koja je prošla na njenu desnu stranu (W_{d}) u odnosu na ukupnu količinu vode (W_{uk}) apsorbovane filter papirima predstavlja vrednost distribucije vode na levoj strani (naličju) pletenine (WD_{L}) prema relaciji (2):

$$WD_{\mathrm{L}} = \frac{W_{\mathrm{d}}}{W_{\mathrm{uk}}} \qquad (2)$$

Za određivanje vrednosti distribucije vode na desnoj strani pletenine (WD_{D}) procedura se ponavlja, s tim što je uzorak pletenine u ovom slučaju okrenut desnom stranom (licem) na gore i na nju je naneta voda. Opisana procedura je za sve pletenine ponovljena na po tri uzorka tako da dobijene vrednosti WD_{L} (distribucija vode na levoj strani pletenine) i WD_{D} (distribucija vode na desnoj strani pletenine) predstavljaju srednje vrednosti tri merenja.

U svrhu analize sposobnosti pletenina da propuštaju vodenu paru i tečnost izračunati su gustina i poroznost DL pletenina. Gustina pletenine, δ (kg m^{-3}), određena je odnosom njene površinske mase i debljine. Ukupna poroznost pletenine, P (%), definisana kao ukupna količina vazduha u pletenini (između i unutar pređe) izračunata je prema relaciji (3), gde je ρ (kg m^{-3}) specifična gustina vlakna:

$$P = 100 - 100\frac{\delta}{\rho} \qquad (3)$$

Statistička značajnost eksperimentalnih rezultata analizirana je ANOVA statistikom (analiza varijanse).

Rezultat ovog testa sastoji se od srednjih vrednosti i varijansi ispitivanih uzoraka, vrednosti statistike (*F*), kritične vrednosti statistike (F_{crit}), kao i nivoa značajnosti (*P*) statistike *F*. ANOVA statistikom se porede srednje vrednosti eksperimentalnih rezultata, pri čemu se u slučaju kada je $F > F_{crit}$, uz uslov da je nivo značajnosti statistike (*P*) manji od praga značajnosti ($\alpha = 0,05$), zaključuje da uzorci ne potiču iz iste populacije, odnosno potvrđuje se uticaj testiranog faktora.

REZULTATI I DISKUSIJA

Svojstva pređa

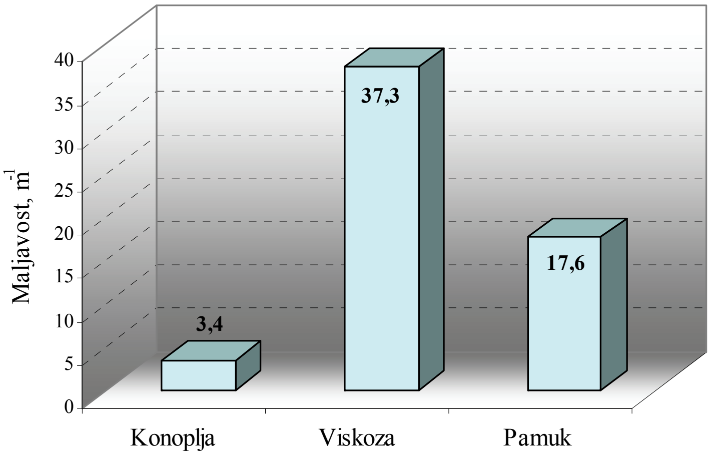
U okviru ovog istraživanja geometrija površine pređe je ocenjena pomoću parametra – maljavost pređe, koja je definisana kao broj malja na jedinici dužine pređe. Za pređe upotrebljene u okviru ovog rada vrednosti maljavosti prikazane su na slici 1 [22]. Viskozna pređa se odlikuje najvećom maljavošću, dok je pređa od konoplje okarakterisana najmanjom maljavošću.

Poznato je da pređe proizvedene tehnikom rotorskog (OE) predenja imaju rastresitu strukturu sa smanjenom gustinom pakovanja vlakana uz prisustvo karakterističnih ovojnih vlakana i površinu koja je sačinjena od vlaknastih petlji. Ovakve pređe sadrže veliku količinu vazduha za razliku od pređa proizvedenih konvencionalnim tehnikama (prstenasto predenje), koje se karakterišu kompaktnom strukturom (veća gustina pakovanja vlakana) i štrčećim krajevima vlakana na površini pređe. U okviru pređa upotrebljenih za izradu DL pletenina, pamučna pređa je proizvedena po rotorskom postupku predenja, dok su viskozna pređa i pređa od konoplje proizvedene na konvencionalnim prstenastim predilicama. Razlika u maljavosti viskozne pređe i pređe od konoplje se može pripisati uticaju deformacionih svojstava ovih vlakana. Naime, pretpostavlja se da mala gipkost vlakana konoplje usporava migraciju vlakana prilikom formiranja pređe čime je sprečeno intenzivnije oslobađanje krajeva vlakana u površinskom sloju pređe.

Svojstva pletenina

Celulozna vlakna se generalno smatraju sirovinom sa visokim potencijalom u pogledu sposobnosti upravljanja vlagom, što se pripisuje prisustvu lumena u unutrašnjosti vlakna i njihovoj hidrofilnoj prirodi (površini). Sa druge strane, ranija istraživanja su pokazala da struktura tekstilnog materijala, pre svega debljina i poroznost, mogu često da imaju presudnu ulogu kada se radi o sposobnosti propuštanja vodene pare i tečnosti. Zbog toga se u okviru ovog istraživanja pristupilo analizi gustine i poroznosti ispitivanih DL pletenina. Rezultati, dati u tabeli 3 ukazuju na izvesne razlike između pletenina uprkos činjenici da su one bile proizvedene na istoj mašini sa unapred zadatim i kontrolisanim parametrima. Ispoljene razlike u gustini i poroznosti pletenina posledica su manjih razlika u konstrukcionim karakteristikama, pre svega debljini i površinskoj gustini (tabela 2). Uočene male razlike u konstrukcionim karakteristikama pletenina posledica su prethodno opisanih razlika u geometriji upotrebljenih celuloznih pređa.

Pamučna pletenina je okarakterisana najvećom poroznošću, što se pripisuje rastresitoj OE pamučnoj pređi koja u sebi sadrži veću količinu vazduha uključenog između vlakana u poređenju sa konvencionalno predenom viskoznom i konopljinom pređom. Pletenina od konoplje i konoplja+viskoza pletenina se odlikuju najmanjom poroznošću, što je posledica njihove povećane gustine (tabela 3) kao i usporene migracije vlakana konoplje prilikom formiranja pređe. Sa aspekta sposobnosti upravljanja vlagom, pored ukupne poroznosti tekstilnog materijala, od velike važnosti je slobodna otvorena površina materijala koju čine makropore – otvori između pređa u tkanini ili pletenini. Praktično se može reći da se difuzija vodene pare kroz otvorene pore (makropore) tekstilnog materijala odvija



Slika 1. Maljavost pređa od kojih su izrađene DL pletenine.
Figure 1. Hairiness of the yarns used for production of the plain knitted fabrics.

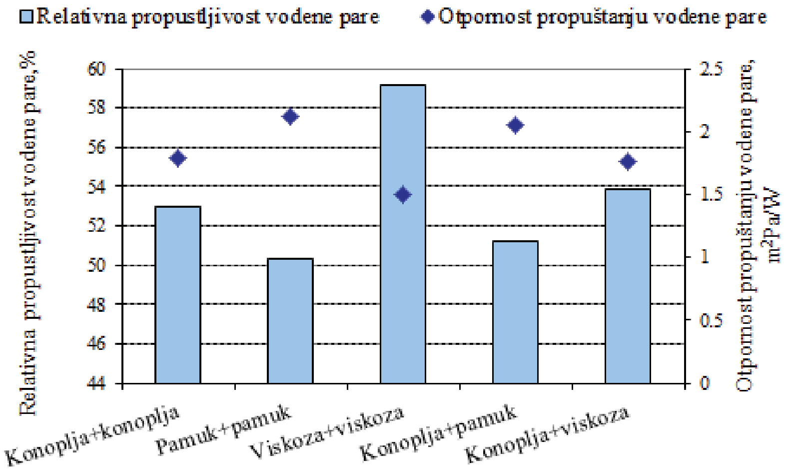
Tabela 3. Fizička svojstva pletenina
Table 3. Physical properties of the knitted fabrics

Sirovinski sastav	Gustina, g cm^{-3}	Poroznost, %	Propustljivost vazduha, m^3 m^{-2} min^{-1}
Konoplja+konoplja	0,398	74,0	64,7
Pamuk+pamuk	0,327	78,2	34,8
Viskoza+viskoza	0,353	76,5	53,7
Konoplja+pamuk	0,353	76,4	46,5
Konoplja+viskoza	0,387	74,2	55,3

nesmetano. Pored toga, kapilarno kvašenje materijala je uslovljeno veličinom i oblikom makropora. Polazeći od činjenice da se strujanje vazduha kroz tekstilni materijal u najvećoj meri dešava kroz makropore, za kvalitativnu ocenu makroporoznosti DL pletenina poslužio je parametar – propustljivost vazduha. Propustljivost vazduha DL pletenina, određena prema standardu ISO 9237 (*Textiles – Determination of the permeability of fabrics to air*), prikazana je u tabeli 3 [23]. Pamučna pletenina, okarakterisana najvećom ukupnom poroznošću, ispoljila je najmanju sposobnost propuštanja vazduha. Rastresita OE pamučna pređa je doprinela većoj ukupnoj poroznosti pletenine, ali je istovremeno zatvorila makropore u pletenini i redukovala propustljivost vazduha. Iako najmanje porozna, pletenina od konoplje je ispoljila najveću propustljivost vazduha. To se pripisuje kompaktnoj (velikoj gustini pakovanja vlakana) pređi od konoplje što je omogućilo stvaranje većeg međuprostora između pređa u pletenini, te veću propustljivost vazduha. Kod pletenina na bazi konoplje (konopljina pređa je jedna od komponentnih pređa) došlo je do stvaranja veće slobodne površine zahvaljujući kompaktnoj pređi od konoplje, pa je propustljivost vazduha ovih pletenina veća u odnosu na odgovarajuće homogene varijante.

Propustljivost vodene pare ili sposobnost materijala da prenosi vodenu paru definiše se kao količina vodene pare koja za određeno vreme prođe kroz jedinicu površine materijala kao rezultat gradijenta pritiska između dve površine materijala. U pogledu toplotnog komfora prednost se daje tekstilnim materijalima sa većom propustljivošću vodene pare. S obzirom na to da je ispitivanje propustljivosti vodene pare sprovedeno u stacionarnim uslovima (odsustvo temperaturnog gradijenta i konstantan gradijent koncentracije vlage), analiza dobijenih rezultata obuhvatila je mehanizme prenosa vodene pare molekulskom difuzijom kroz vlakno, difuzijom po površini vlakna i difuzijom kroz otvorene pore pletenina. Rezultati ispitivanja propustljivosti vodene pare DL pletenina, predstavljeni na slici 2, ukazuju na određene razlike između pletenina. Statistička analiza je pokazala da uočene razlike u pogledu propustljivosti vodene pare nisu slučajnog karaktera (F(13,05) > F_{crit}(3,48) za $P(0{,}56\times10^{-3}) < \alpha(0{,}05)$).

U okviru ispitivanih DL pletenina izdvojila se viskozna pletenina sa najmanjom otpornošću, odnosno najvećom propustljivošću vodene pare. Najmanju sposobnost propuštanja vodene pare ispoljila je pamučna pletenina, koja je prethodno ocenjena kao pletenina sa najmanjom slobodnom otvorenom površinom (tabela 3, propustljivost vazduha). Distribucija pora u pamučnoj pletenini u korist mikropora (pore između vlakana u pređi) prouzrokovala je difuziju vodene pare naizmenično kroz gasovitu i polimernu fazu, što je usporilo

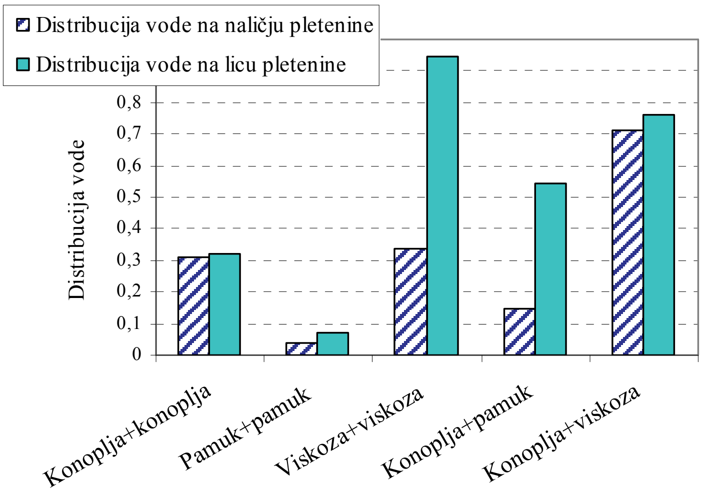


Slika 2. Propustljivost vodene pare DL pletenina.
Figure 2. Water vapour permeability of the plain knitted fabrics.

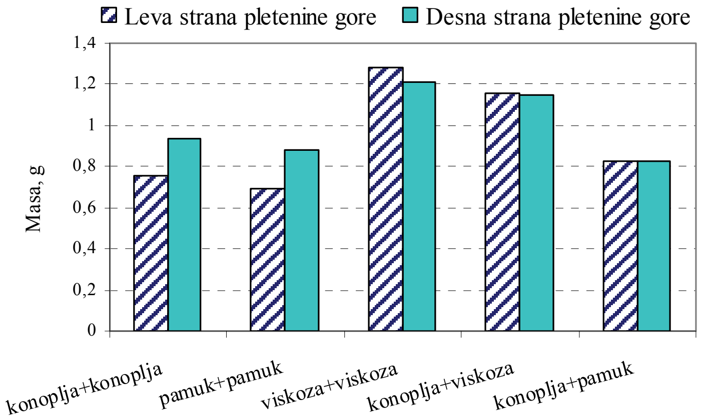
prenos vodene pare kroz materijal. Naime, znatno niži koeficijent difuzije vodene pare kroz celulozno vlakno (10^{-7} cm^2 s^{-1}) [3] u odnosu na vazduh (0,239 cm^2 s^{-1}) ograničio je difuziju vlage unutar vlakna i izvan njega. Sa druge strane, sposobnost pletenine od konoplje da propušta vodenu paru je manja u odnosu na viskoznu pleteninu uprkos tome što je konopljina pletenina okarakterisana većom makroporoznošću. Ovakav rezultat ukazuje na mogućnost da je kod viskozne pletenine intenzivirana površinska difuzija adsorbovanih molekula vlage kao posledica povećanja specifične površine pletenine usled izražene maljavosti viskozne pređe. Ponašanje pletenina kod kojih je konopljina pređa bila jedna od komponenti u pogledu propustljivosti vodene pare reflektuje uticaj obe komponente. Tako je kod konoplja+pamuk pletenine uočeno povećanje propustljivosti vodene pare u odnosu na pamučnu pleteninu zahvaljujući izvesnom povećanju otvorene slobodne površine uvođenjem kompaktne pređe od konoplje.

Poznato je da je sposobnost kvašenja tekstilnih materijala uslovljena vrstom vlakana, strukturom i svojstvima pređe i strukturom tekstilne površine. Eksperimentalni materijal koji je upotrebljen u ovom istraživanju omogućio je ocenu uticaja vrste vlakana i geometrije pređe na sposobnost kvašenja DL pletenina. Pored toga, primenjena metoda je omogućila sagledavanje specifičnosti strukture DL pletenina u pogledu karakteristika kvašenja. Sposobnost DL pletenina da propuštaju tečnost ocenjena je parametrima koji opisuju distribuciju vode na levoj (naličju) i desnoj (licu) strani pletenine. Rezultati su prikazani na slici 3.

Test distribucije vode na levoj i desnoj strani pletenine daje odgovor na pitanje koliko tečnosti svaka od ispitivanih pletenina može da propusti. Veća vrednost parametra pokazuje da veća količina tečnosti prolazi kroz pleteninu, odnosno ukazuje na veću sposobnost kapilarnog kvašenja pletenine. Najmanju sposobnost prenosa tečnosti pokazala je pamučna pletenina što se, prema teoretskim saznanjima, moglo i pretpostaviti. Naime, ova činjenica se može pripisati maloj makroporoznosti pamučne pletenine, što je potvrđeno vrednostima propustljivosti vazduha (tabela 3), i dobro poznatim apsorpcionim svojstvima pamučnih vlakana. Sa druge strane, najveća sposobnost prenosa tečnosti uočena je kod viskozne pletenine i konoplja+viskoza pletenine, iako je makroporoznost ovih pletenina niža u poređenju sa pleteninom od konoplje. U svrhu tumačenja ovih rezultata, pristupilo se izračunavanju mase vode koja se prilikom sprovođenja testa zadržala u pletenini. Ova vrednost se dobija kada se od mase vode nanete na uzorak pletenine oduzme ukupna masa vode apsorbovane filter papirima. Na grafikonu prikazanom na slici 4 može se uočiti da su u testu distribucije vode pletenine ispoljile različitu sklonost ka zadržavanju vode, što je potvrđeno analizom varijanse u slučaju kada je voda naneta na lice pletenine ($F(3{,}49) > F_{\text{crit}}(3{,}25)$ za $P(0{,}042) < \alpha(0{,}05)$), i u slučaju kada je naličje pletenine bilo u kontaktu sa vodom ($F(12{,}13) > F_{\text{crit}}(3{,}25)$ za $P(0{,}75\times10^{-3}) < \alpha(0{,}05)$). Uočava se da su viskozna i konoplja+viskoza pletenina zadržale najveću količinu vode. Razlog tome je poznat povećani afinitet viskoznih vlakana ka apsorpciji vode. Pored toga, dosadašnja istraživanja su pokazala da, iako se makropore u tekstilnom materijalu brzo „pune“ tečnošću, mikropore su odgovorne za dostizanje maksimalne visine tečnosti, ili u konkretnom slučaju, mikropore pomažu da tečnost „izbije“ na suprotnu površinu pletenine. Manja otvorena slobodna površina viskozne i konoplja+viskoza pletenine, uz veću ukupnu poroznost, navode na zaključak da su mikropore u većoj meri zastupljene kod ovih pletenina u poređenju sa pleteninom od konoplje. Otuda se kod pletenine od konoplje, uprkos njenoj najvećoj



Slika 3. Sposobnost kapilarnog kvašenja DL pletenina.
Figure 3. Water distribution values of the plain knitted fabrics.



Slika 4. Masa vode koja se zadržala u pletenini u testu distribucije vode.
Figure 4. Water held by a knitted fabric in water distribution test.

makroporoznosti, uočava manja sposobnost prenosa tečnosti u odnosu na pletenine koje sadrže viskoznu pređu.

Statistička analiza (ANOVA) rezultata kapilarnog kvašenja pletenina dobijenih u testu distribucije vode potvrdila je prethodno tumačenje u slučaju kada su pletenine bile ispitivane sa licem na gore, odnosno kada je desna strana pletenine bila u kontaktu sa vodom ($F(7{,}04) > F_{\mathrm{crit}}(3{,}48)$ za $P(5{,}8\times10^{-3}) < \alpha(0{,}05)$). S obzirom da razlike u sposobnosti prenosa vode, uočene između uzoraka pletenina kada je njihovo naličje bilo u kontaktu sa vodom, nisu potvrđene statističkom analizom ($P(0{,}16) > \alpha(0{,}05)$), nameće se potreba daljeg ispitivanja uz povećanje broja merenja po pletenini.

ZAKLJUČAK

Sprovedenim istraživanjima je potvrđeno da distribucija pora u pleteninama predstavlja ključni parameter koji definiše njihovu sposobnost da propuštaju vodenu paru i tečnost. Pri tome treba imati u vidu da je distribucija pora u okviru eksperimentalnog materijala bila uslovljena deformacionim svojstvima upotrebljenih prirodnih i regenerisanih celuloznih vlakana, kao i primenjenim tehnikama izrade pređa. Iako je generalno potvrđena analogija između sposobnosti upravljanja vlagom i otvorene slobodne površine pletenina, pokazalo se da njihova korelacija nije uvek jednostavna. Interakciju makroporoznosti pletenina i njihove sposobnosti da propuštaju vodenu paru komplikuje fenomen difuzije molekula vlage duž površine vlakana odnosno pređe, dok vlakna svojom prirodom mogu da modifikuju sposobnost kapilarnog kvašenja pletenina.

Dobijeni rezultati ukazuju na mogućnost da se pažljivim izborom sirovine (vlakana) i projektovanjem strukture pređe mogu dobiti pletenine sa unapred definisanom sposobnošću upravljanja tečnostima. Kako bi se dobile preciznije smernice u tom smislu, neophodna su dalja istraživanja uz proširenje eksperimentalnog materijala u pravcu upotrebe različitih vrsta vlakana i različitih struktura pređa i pletenina.

Zahvalnica

Zahvaljujemo se Ministarstvu prosvete, nauke i tehnološkog razvoja Republike Srbije za finansiranje projekta OI171029.

LITERATURA

[1] V.T. Bartels, in: G. Song (Ed.), Improving Comfort in Clothing, Woodhead Publishing Ltd., Cambridge, 2011, pp. 385–411.
[2] S. Stanković, Komfor odeće, Zadužbina Andrejević, Tehnološko–metalurški fakultet, Beograd, 2012, pp. 37–43.
[3] H.N. Yoon, A.Buckley, Improved comfort polyester: Part I: Transport properties and thermal comfort of polyester/cotton blend fabric, Text. Res. J. **54** (1984) 289––298.
[4] M.M. Adler, W.K. Walsh, Mechanisms of transient moisture transport between fabrics, Text. Res. J. **54** (1984) 334–343.
[5] B. Miller, D.B. Clark, Liquid transport trough fabrics, wetting and steady-state flow, Text. Res. J. **48** (1978) 150–155.
[6] E. Kissa, Wetting and wicking, Text. Res. J. **66** (1996) 660–668.
[7] R.K. Nayak, S.K. Punj, K.N. Chaterjee, Comfort properties of suiting fabrics, Ind. J. Fiber Text. Res. **34** (2009) 122––128.
[8] N. Ozdil, A. Marmarali, S.D. Kretzschmar, Effect of yarn properties on thermal comfort of knitted fabrics, Int. J. Therm. Sci. **46** (2007) 1318–1322.
[9] A. Majumdar, S Mukhopadhyay, R. Yadav, Thermal properties of knitted fabrics made from cotton and regenerated bamboo cellulosic fibers, Int. J. Therm. Sci. **49** (2010) 2042–2048.
[10] W. Zhong, X. Ding, Z.L. Tang, Analysis of fluid flow through fibrous structures, Text. Res. J. **72** (2002) 751––755.

[11] S. Stanković, K. Asanović, B. Pejić, Karakteristike kvašenja pletenina na bazi konoplje namenjenih za medicinske svrhe, Tekstilna industrija **54** (2006) 24–29.

[12] M. Novaković, M. Bizjak, S. Stanković, Uticaj strukture pređe na propustljivost vodene pare glatkih pletenina, Savremene tehnologije **2** (2013) 15–20.

[13] A. Perwuelz, M. Casetta, C. Caze, Liquid organisation during capillary rise in yarns- influence of yarn torsion, Polym. Test. **20** (2001) 553–561.

[14] C.I. Su, J.X. Fang, X.H. Chen, W.Y. Wu, Moisture absorption and release of profiled polyester and cotton, composite knitted fabrics, Text. Res. J. **77** (2007) 764–769.

[15] R. Fangueiro, A. Fligueiras, F. Soutinho, X. Meidi, Wicking behavior and drying capabillity of functional knitted fabrics, Text. Res. J. **80** (2010) 1522–1530.

[16] SRPS ISO 2060, Pređa u namotajima – Određivanje linearne gustine – Metoda povesma, 1995.

[17] SRPS ISO 2061, Tekstil – Određivanje uvoja u pređi – Metoda direktnog brojanja, 1994.

[18] SRPS EN ISO 5084, Tekstil – Određivanje debljine tekstila i tekstilnih proizvoda, 2013.

[19] A. Koblyakov, in A. Koblyakov (Ed.), Laboratory Practice in the Study of Textile Materials, Mir Publisher, Moscow, 1989, pp. 254–262.

[20] Sensora Instruments & Consulting, Manual of use of the instrument.

[21] ISO 11092, Textiles – Physiological effects – Measurement of the thermal and water-vapour resistance.

[22] S. Stanković, Uticaj strukture pređa na bazi agroceluloznih vlakana na njihovu dalju tekstilnu transformaciju i upotrebna svojstva, Doktorska disertacija, Tehnološko–metalurški fakultet, Beograd, 2009.

[23] S. Stanković, D. Popović, G. Poparić, Thermal properties of textile fabrics made of natural and regenerated cellulose fibers, Polym. Test. **27** (2008) 41–48.

SUMMARY

MOISTURE MANAGEMENT PROPERTIES OF PLAIN KNITTED FABRICS MADE OF NATURAL AND REGENERATED CELLULOSE FIBRES

Milada S. Novaković[1,2], Lana S. Putić[2], Matejka Bizjak[3], Snežana B. Stanković[2]

[1]High Technical Colledge of Professional Studies, Zrenjanin, Serbia
[2]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia
[3]University of Ljubljana, Facuty of Natural Sciences and Engineering, Ljubljana, Slovenia

(Scientific paper)

Moisture management is a complicated process which is known to be influenced by a variety of fabric characteristics such as fibre nature (hydrophilic or hydrophobic), porosity and thickness. There are different aspects of the moisture management properties of textile materials since water transport in textile materials can be in the form of liquid and vapour. The ability of textile materials to transfer water vapour allows the human body to keep thermal balance due to evaporation. With stronger physical activity of a person when the body produces a large amount of heat, the skin perspiration increases (in order to regulate the body temperature) and liquid sweat should be taken from the skin, otherwise it will worsen the sense of comfort. The aim of this research was to investigate the factors influencing moisture management properties of plain knitted fabrics at the three scale levels, *i.e.*, microscopic (fibre type), mesoscopic (yarn geometry) and macroscopic (fabric porosity) levels. Plain knitted fabrics were produced from the two-assembled hemp, cotton and viscose yarns under controlled conditions so as to be comparable in basic construction characteristics, but varying in yarns geometry. Evaporative resistance test reflecting vapour transport and water distribution test reflecting liquid transport in the knitted fabrics were conducted. To determine the statistical importance of the results, analysis of variance (ANOVA) was applied. As a consequence of the geometry and deformation behaviour of the fibres used and spinning techniques applied, the yarns differed in both packing density and surface geometry, thus determining the pore distribution. Due to loose structure of the cotton yarn, the cotton knitted fabric was characterised by the lowest free open surface (macroporosity) exhibiting the lowest both water vapour and liquid permeability. Although having the highest macroporosity, the water vapour and liquid transport capability of the hemp knitted fabric was lower than that of the viscose knit. The best moisture management properties of the viscose knitted fabric were resulted from viscose affinity for water absorption and increased surface area of the viscose yarn. The results obtained proved that variations in any of the hierarchical structure levels can modify moisture transport ability of textile fabrics. Therefore, the moisture management properties of textile materials can be guided in a desired direction by the appropriate selection of fibres and careful design of yarn structure.

Keywords: Knitted fabric • Moisture management • Water vapor permeability • Water distribution • Porosity • Yarn hairiness

Biosinteza komponenti antifungalnog delovanja prema *Aspergillus* spp. primenom *Streptomyces hygroscopicus*

Jelena M. Dodić[1], **Zorana Z. Rončević**[1], **Jovana A. Grahovac**[1], **Bojana Ž. Bajić**[1], **Olivera S. Korolija**[2]

[1]*Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, Srbija*
[2]*Hemofarm a.d., Vršac, Srbija*

Izvod

Tokom skladištenja jabuka dolazi do njihovog propadanja usled infekcija izazvanih fitopatogenim gljivama. U zaštiti bilja sve više se koriste biološki preparati kako bi se smanjili štetni efekti primene hemijskih sredstava. Cilj ovog rada je optimizacija sastava podloge za proizvodnju antifungalnih agenasa primenom soja *Streptomyces hygroscopicus*. Antifungalno delovanje kultivacione tečnosti prema *Aspergillus* spp. procenjeno je, *in vitro*, difuzionom disk metodom. Izvršena je optimizacija sastava hranljive podloge i definisano je da pri početnim sadržajima glukoze, sojinog brašna i fosfata od 47,77, 24,54 i 0,98 g/l, redom, kultivaciona tečnost formira najveću zonu prosvetljenja (40,93 mm). Pored toga, urađena je i optimizacija sa dodatnim ciljem minimizacije utrošaka sastojaka hranljive podloge što omogućava dalju tehno-ekonomsku analizu procesa u industrijskim uslovima.

Ključne reči: *Aspergillus spp., antifungalna jedinjenja, Streptomyces hygroscopicus, optimizacija podloge.*

NAUČNI RAD

UDK 634.11–156.3:66

Hem. Ind. **69** (2) 201–208 (2015)

doi: 10.2298/HEMIND130416009D

Jabuka je jedna od najzastupljenijih voćnih vrsta u svetu i njeni plodovi su dostupni na tržištu tokom cele godine. Zaštita plodova jabuke od prouzrokovača truleži, obavlja se u voćnjaku, najkasnije dve nedelje pre berbe, dok se tretiranja prilikom i u toku skladištenja ne primenjuju zbog karence i nedostatka zdravstveno bezbednih preparata. Razvoj novih biofungicida omogućio bi zaštitu plodova jabuke i tokom skladištenja što bi imalo pozitivan efekat kako sa zdravstvenog tako i sa ekonomskog aspekta [1,2].

Vrste roda *Aspergillus*, pored vrsta rodova *Alternaria*, *Fusarium, Colletotrichum* i *Penicillium*, predstavljaju izazivače bolesti jabuka [3]. Posledice inficiranja jabuka ovim fitopatogenima su truljenje, pigmentacija, promena boje, mirisa i ukusa. S obzirom da tokom patogeneze *Aspergillus* vrste proizvode različite mikotoksine, najveći problem infekcije je akumulacija toksičnih proizvoda njihovog metabolizma u plodovima jabuke. Proizvodi dobijeni preradom kontaminiranih jabuka su, takođe, izvor mikotoksina jer je veliki broj njih stabilan u ovim procesima [4,5]. Zbog toga je veoma značajno zaštititi jabuke od infekcija izazvanih *Aspergillus* vrstama.

U zaštiti bilja dominiraju hemijske mere borbe, odnosno korišćenje hemijskih sredstava ili pesticida. Primene klasičnih pesticida imaju za posledicu kontaminaciju proizvoda, zagađenje životne sredine, poremećaj prirodne ravnoteže i izumiranje nekih živih organizama u ekosistemu. U cilju očuvanja prirodne ravnoteže i proizvodnje zdravije hrane, vrši se supstitucija klasičnih agrohemikalija sa prirodnim proizvodima koji su ekološki prihvatljivi, a poseduju pesticidne osobine [6,7]. Mikroorganizmi su glavni izvori bioaktivnih agenasa. Proizvodi njihovog metabolizma štite biljke delujući antagonistički na izazivače bolesti pri čemu su bezopasni za ljude i ekološki bezbedni [8]. Poznati biotehnološki procesi imaju veliki potencijal za proizvodnju jedinjenja sa antifungalnim delovanjem. U dosadašnjim istraživanjima kao proizvodni mikroorganizam često su primenjivane bakterije roda *Streptomyces* [9]. One mogu da sintetišu veliki broj agenasa sa antagonističkim delovanjem prema fitopatogenim *Aspergillus* vrstama [9–14]. S obzirom na to da su pomenute komponente sekundarni proizvodi metabolizma streptomiceta, veoma je važno formulisati sastav hranljive podloge tako da se favorizuje njihova sinteza uz ograničen rast biomase. Varijacije u sastavu hranljive podloge i procesnih uslova značajno utiču na to koja će antifungalna komponenta biti sintetisana, ali i na njen prinos. Stoga su pravilan izbor izvora ugljenika, azota, fosfata i mineralnih soli i tačno definisanje njihovih koncentracija, kao i vrednosti pH, temperature i trajanja procesa veoma bitni za povećanje prinosa željenog biofungicida [15,16].

Klasičan pristup eksperimentalnom radu podrazumeva ispitivanje uticaja pojedinačnih faktora na neki proces. Iako ova tehnika zahteva dosta napora i vremena, najveći nedostatak njene primene je što ne obuhvata interakcije faktora te stoga ne prikazuje ukupan uticaj faktora na proces. Navedeni nedostaci mogu se eliminisati statističkim planiranjem eksperimenata [17].

Prepiska: Z. Rončević, Tehnološki fakultet, Univerzitet u Novom Sadu, Bulevar cara Lazara 1, 21000 Novi Sad, Srbija.
E-pošta: ron@uns.ac.rs

Rad primljen: 16. april, 2013
Rad prihvaćen: 5. februar, 2014

Postupak odzivne površine (RSM – *response surface methodology*) je poslednjih godina postao najpopularnija tehnika za kreiranje eksperimentalnog plana, modelovanje i opisivanje interakcija velikog broja faktora zbog čega je posebno pogodan za rešavanje optimizacionih problema. Budući da na mikrobiološku proizvodnju antifungalnih komponenti utiče veliki broj faktora, obično je veoma teško utvrditi koji od njih ima najveći uticaj. Stoga se RSM sve više primenjuje za optimizaciju biotehnoloških procesa proizvodnje ovih komponenti [18,19].

Cilj ovog rada je optimizacija sastava hranljive podloge, u pogledu sadržaja glukoze, sojinog brašna i fosfata, za proizvodnju agenasa sa antifungalnim delovanjem prema *Aspergillus* spp. Za biosintezu upotrebljen je soj *Streptomyces hygroscopicus* izolovan iz prirodnog okruženja.

EKSPERIMENTALNI DEO

Mikroorganizmi

Za ispitivanje antifungalne aktivnosti, kao test mikroorganizmi, primenjene su dve vrste fitopatogenih gljiva, *Aspergillus oryzae* i *Aspergillus niger*, koje su izolovane sa plodova jabuka sa simptomima truljenja. Za biosintezu antifungalnih komponenti upotrebljen je soj *Streptomyces hygroscopicus* izolovan iz uzorka zemljišta sa područja Novog Sada, Srbija, koji je identifikovan na osnovu morfoloških i fizioloških karakteristika [20,21]. Svi korišćeni mikroorganizmi čuvaju se u zbirci kultura laboratorije za biohemijsko inženjerstvo, Tehnološkog fakulteta, Novi Sad, Srbija.

Hranljive podloge

Za umnožavanje proizvodnog mikroorganizma koriste se hranljive podloge čiji sastav omogućava maksimalan rast biomase. U okviru ovog istraživanja za pripremu inokuluma korišćena je hranljiva podloga sledećeg sastava (g/l): glukoza (15,0), sojino brašno (10,0), $CaCO_3$ (3,0), NaCl (3,0), $MgSO_4$ (0,5), $(NH_4)_2HPO_4$ (0,5) i K_2HPO_4 (1,0).

U skladu sa definisanim ciljem rada i eksperimentalnim planom za biosintezu antifungalnih komponenti korišćeno je više podloga u kojima su varirani sadržaji glukoze (10, 30 i 50 g/l), sojinog brašna (5, 15 i 25 g/l) i fosfata (0,5, 1,5 i 2,5 g/l). Fosfati su dodati u obliku $(NH_4)_2HPO_4$ i K_2HPO_4 u odnosu 1:2. Ostale komponente podloge su (g/l): $CaCO_3$ (3,0), NaCl (3,0) i $MgSO_4$ (0,5).

Vrednost pH hranljivih podloga korigovana je na 7,2 ± 0,1. Sterilizacija pripremljenih podloga je izvedena u autoklavu pri temperaturi od 121 °C i pritisku od 2,1 bar u trajanju od 20 min.

Priprema inokuluma

Proizvodni mikroorganizam iz zbirke kultura osvežen je na agarizovanoj podlozi odgovarajućeg sastava, u aerobnim uslovima, stacionarnom kultivacijom, na temperaturi od 27 °C (mikrobiološki laboratorijski termostat), u toku 48 h. Osvežena kultura umnožena je postupkom dvostrukog pasažiranja u količini dovoljnoj za inokulaciju podloge za biosintezu. Druga pasaža zasejana je sa 10 zapr.% inokuluma proizvedenog prvim pasažiranjem. Inkubiranje svake pasaže izvedeno je u aerobnim uslovima, uz eksterno mešanje (laboratorijska tresilica, 150 o/min), na temperaturi od 27 °C u toku 48 h.

Uslovi biosinteze

Eksperimenti su izvedeni u erlenmajerima radne zapremine 100 ml. Hranljive podloge za biosintezu inokulisane su sa 10 zapr.% inokuluma pripremljenog po opisanom postupku. Biosinteza antifungalnih komponenti u trajanju od 7 dana izvedena je u aerobnim uslovima pri temperaturi od 27 °C i brzini mešanja 150 o/min, koja je obezbeđena postavljanjem erlenmajera na mikrobiološku laboratorijsku tresilicu u termostatu.

Analitičke metode

Po završenoj biosintezi kultivacione tečnosti su centrifugirane pri 10000*g* u trajanju od 10 min (Eppendorf Centrifuge 5804, Germany). U skladu sa zahtevima planiranih analiza u daljem radu korišćen je supernatant kultivacione tečnosti.

Sadržaj šećera određen je metodom po Mileru [22], dok je za određivanje sadržaja ukupnog azota korišćena metoda po Kjeldalu [23].

Ispitivanje antifungalnog delovanja

Biosinteza komponenti antifungalnog delovanja prema *Aspergillus oryzae* i *Aspergillus niger* ispitana je, *in vitro*, difuzionom disk metodom [24]. Test mikroorganizmi su osveženi na podlozi na kojoj se uobičajeno gaje *Aspergillus* vrste (Sabouraud maltozni agar), a zatim je nekoliko kolonija osveženih kultura suspendovano u 10 ml sterilnog fiziološkog rastvora. Ove suspenzije su razblaživane do postizanja koncentracije od 10^5 spora/ml. Sterilna komercijalna podloga temperirana je na 50 °C nakon čega je u nju dodata suspenzija spora test mikroorganizma u odnosu 9:1. Nakon želiranja podloga u Petri pločama postavljeni su sterilni diskovi (HiMedia, India) na koje je nanošeno po 10 μl prethodno koncentrovanih uzoraka. Koncentrovanje uzoraka izvedeno je njihovim uparavanjem na deseti deo mase rotacionim vakuum uparivačem (Ika-werke, Staufen) na 45 °C. Inokulisane Petri ploče inkubirane su 48 h na 30 °C nakon čega je odgovarajućim lenjirom (HiAntibiotic ZoneScale, Himedia®) izmeren prečnik nastalih zona prosvetljenja.

Plan eksperimenta i obrada podataka

Izbor odgovarajućeg eksperimentalnog plana ključan je korak u primeni postupka odzivne površine [25]. U okviru ovih istraživanja korišćen je Box–Behnken eksperimentalni plan sa tri faktora na tri nivoa i tri ponavljanja u centralnoj tački. U tabeli 1 prikazan je Box–Behnken plan eksperimenta i varirane vrednosti sadržaja glukoze, sojinog brašna i fosfata koje su korišćene u ovom radu.

Za opis odzivne funkcije Y (prečnik zone prosvetljene prema *Aspergillus* spp. (mm), rezidualni sadržaj glukoze (g/l), rezidualni sadržaj ukupnog azota (g/l)) primenjen je polinom drugog reda:

$$Y = b_0 + \sum b_i X_i + \sum b_{ii}^2 X_{ii}^2 + \sum b_{ij} X_i X_j \tag{1}$$

gde je b_0 odsečak, b_i linearni koeficijent, b_{ii} kvadratni koeficijent, a b_{ij} koeficijent interakcije.

Vrednosti odziva prečnika zone prosvetljenja prema *Aspergillus* spp. koje su primenjene za modelovanje predstavljaju srednje vrednosti dobijene iz tri ponovljena eksperimenta za oba ispitivana test mikroorganizma.

Statistička obrada eksperimentalnih podataka i crtanje odzivnih površina urađeni su primenom softverskog paketa Statistica 12.0. Značajnost uticaja svakog od faktora kao i njihovih interakcija određivana je poređenjem *p*-vrednosti za svaki od koeficijenata u regresionoj jednačini. Odzivne površine su crtane tako što je jedan od faktora imao konstantnu vrednost iz centra plana, dok su preostala dva faktora varirana. Za optimizaciju faktora primenjena je metoda željene funkcije, a rađena je u softerskom paketu Design-Expert 8.1.

REZULTATI I DISKUSIJA

Statistička obrada podataka

U skladu sa definisanim ciljem rada i eksperimentalnim planom formulisane su podloge za biosintezu antifungalnih komponenti. Na osnovu podataka prikupljenih iz dostupne naučne literature odabrane su najveća, srednja i najmanja vrednost faktora X_1, X_2 i X_3, odnosno sadržaja glukoze (10, 30 i 50 g/l), sadržaja sojinog brašna (5, 15 i 25 g/l) i sadržaja fosfata (0,5, 1,0 i 2,5 g/l), redom. Rezultati eksperimenata baziranih na Box-Behnken dizajnu fitovani su polinomom drugog reda. Kao odzivi definisani su prečnik zone prosvetljenja prema *Aspergillus* spp. (Y_1), rezidualni sadržaj glukoze (Y_2) i rezidualni sadržaj ukupnog azota (Y_3). Rezultati fitovanja odabranih odziva polinomom drugog reda prikazani su u tabelama 2 i 3.

Za procenu statističke značajnosti svakog od koeficijenata regresione jednačine koristi se *p*-vrednost. Regresioni koeficijenti, pri nivou značajnosti od 95%, su značajni ukoliko je njihova *p*-vrednost manja od 0,05 i takvi su u tabeli 2 naglašeni masnim slovima.

U tabeli 3 prikazani su rezultati analize varijanse modelovanih odziva. Relativno visoke vrednosti (veće od 0,900) koeficijenta determinacije (R^2) ukazuju na veoma dobro fitovanje eksperimentalnih rezultata polinomom drugog reda. Modeli polinoma drugog reda svih posmatranih odziva su značajni pri nivou značajnosti od 95% što potvrđuju *F*-vrednosti od 53,24, 66,99 i 309,92 za prečnik zone prosvetljenja prema *Aspergillus* spp., rezidualni sadržaj šećera i rezidualni sadržaj ukupnog azota, redom.

Tabela 1. Box–Behnken plan eksperimenta i varirane vrednosti parametara
Table 1. Box–Behnken experimental plan and varied values of factors

Redni broj eksperimenta	Kodirane vrednosti			Varirane vrednosti, g/l, za:		
	X_1	X_2	X_3	Glukoza	Sojino brašno	Fosfati
1	–1	–1	0	10	5	1,5
2	1	–1	0	50	5	1,5
3	–1	1	0	10	25	1,5
4	1	1	0	50	25	1,5
5	–1	0	–1	10	15	0,5
6	1	0	–1	50	15	0,5
7	–1	0	1	10	15	2,5
8	1	0	1	50	15	2,5
9	0	–1	–1	30	5	0,5
10	0	1	–1	30	25	0,5
11	0	–1	1	30	5	2,5
12	0	1	1	30	25	2,5
13	0	0	0	30	15	1,5
14	0	0	0	30	15	1,5
15	0	0	0	30	15	1,5

Tabela 2. Koeficijenti regresione jednačine i njihova značajnost za prečnik zone prosvetljenja prema Aspergillus spp. (Y_1), rezidualni sadržaj glukoze (Y_2) i rezidualni sadržaj ukupnog azota (Y_3)
Table 2. Regression equation coefficients and their significance for inhibition zone diameter for Aspergillus spp. (Y_1), residual glucose (Y_2) and residual nitrogen (Y_3)

Odzivi	Y_1		Y_2		Y_3	
Efekti	Koeficijent	*p*-vrednost	Koeficijent	*p*-vrednost	Koeficijent	*p*-vrednost
			Odsečak			
b_0	–0,7812	0,9522	6,4287	0,4435	0,0536	0,5810
			Linearni			
b_1	1,0688	0,0576	–0,1902	0,5149	0,0123	**0,0121**
b_2	–3,0625	0,5131	0,3469	0,5511	–0,0050	0,4678
b_3	27,6249	**0,0247**	–11,4653	0,0885	0,1292	0,0993
			Kvadratni			
b_{11}	–0,0147	0,0551	0,0181	**0,0044**	–0,00019	**0,0066**
b_{22}	–0,5938	0,3603	–0,0016	0,9165	0,00099	**0,0022**
b_{33}	–7,6250	**0,0232**	3,3102	0,0744	–0,03457	0,1025
			Interakcije			
b_{12}	0,1938	**0,0189**	–0,0223	**0,0254**	–0,00001	0,8786
b_{13}	–0,2750	0,0596	0,1700	0,0613	–0,00092	0,3192
b_{23}	0,2500	0,8341	–0,0899	0,5528	–0,00079	0,6540

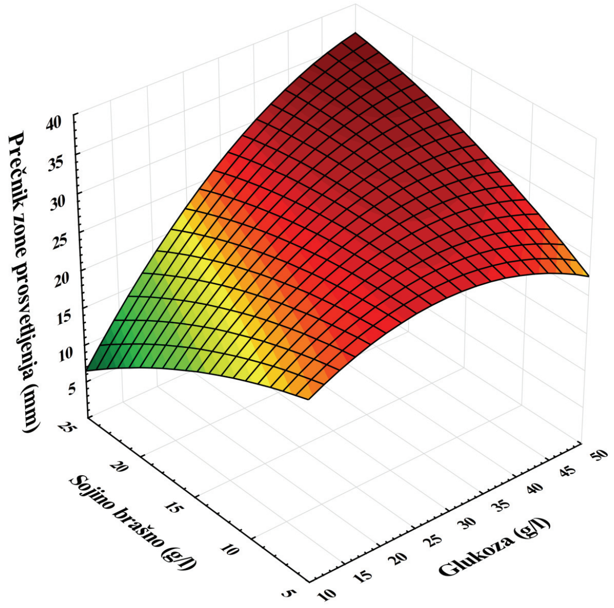
Tabela 3. Analiza varijanse (ANOVA) modelovanih odziva: prečnik zone prosvetljenja prema Aspergillus spp. (Y_1), rezidualni sadržaj glukoze (Y_2) i rezidualni sadržaj ukupnog azota (Y_3); DF – stepen slobode; SS – suma kvadrata; MS – srednja vrednost kvadrata
Table 3. Analysis of variance (ANOVA) of the modelled responses: inhibition zone diameter for Aspergillus spp. (Y_1), residual glucose (Y_2) and residual nitrogen (Y_3); DF – degree of freedom, SS – sum of squares, MS – mean squares

Odziv	Poreklo								
	Rezidual			Model			F	*p*-vrednost	R^2
	DF	SS	MS	DF	SS	MS			
Y_1	5	102,75	20,55	10	10940,25	1094,025	53,24	0,000191	0,919
Y_2	5	39,99	7,99	10	5358,29	535,829	66,99	0,000109	0,962
Y_3	5	0,0055	0,0011	10	3,43	0,343	309,92	0,000002	0,989

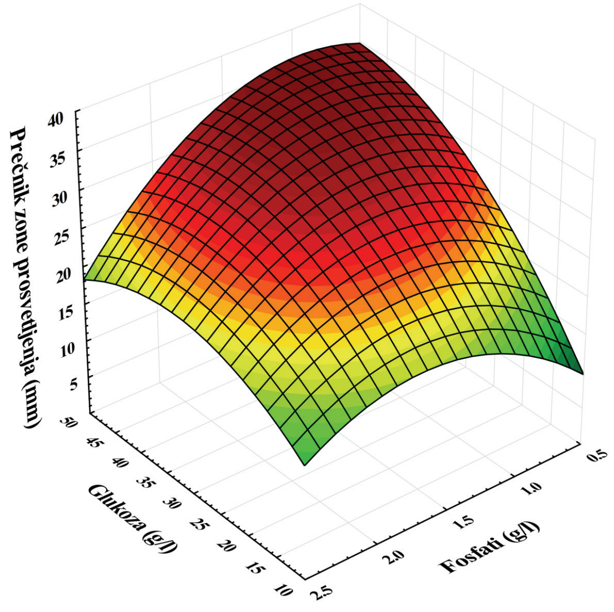
Kako bi se razumele interakcije faktora koji utiču na biosintezu antifungalnih komponenti nacrtane su odzivne površine (slike 1–3). Svaka od njih prikazuje modelom predviđen uticaj dva faktora na proces pri konstantnoj vrednosti trećeg. Sa odzivnih površina moguće je proceniti optimalne vrednosti faktora pri kojima se sintetišu komponente sa najvećim antifungalnim delovanjem [18].

Slika 1 ilustruje modelom predviđen uticaj početnog sadržaja glukoze i sojinog brašna na prečnik zone prosvetljenja prema *Aspergillus* spp. pri konstantnom sadržaju fosfata (1,5 g/l). Analizom prikazanih rezultata se uočava da pri malom početnom sadržaju glukoze porast sadržaja sojinog brašna negativno utiče na sintezu antifungalnih agenasa. Razlog za to je usmeravanje metabolizma proizvodnog mikroorganizma ka sintezi biomase u slučaju prisustva veće količine izvora azota. Kada je rast biomase limitiran dodatkom malih količina sojinog brašna, dalje povećanje sadržaja glukoze nije pokazalo značajan uticaj na produkciju antifungalnih agenasa. Međutim, pri većim početnim sadržajima izvora azota (15–25 g/l) povećanje sadržaja glukoze značajno utiče na posmatrani odziv. Sinergistički efekat sadržaja glukoze i sojinog brašna, na koji ukazuje pozitivan koeficijent interakcije $b_{1,2}$ (tabela 2), ogleda se u porastu prečnika zone prosvetljenja pri većim sadržajima obe komponente. U podlogama sa velikim početnim koncentracijama izvora ugljenika i azota za kratko vreme dostiže se maksimalan rast biomase, nakon čega se metabolizam mikroorganizma usmerava ka produkciji većih količina antifungalnih komponenti. Maksimalan prečnik zone prosvetljenja od oko 35 mm predviđen je u opsegu početnog sadržaja glukoze 45–50 g/l i sojinog brašna 20–25 g/l.

Na slici 2 prikazan je modelom predviđen uticaj početnog sadržaja glukoze i fosfata na prečnik zone prosvetljenja prema odabranom test mikroorganizmu pri sadržaju sojinog brašna od 15 g/l. Pri početnim koncentracijama fosfata 1,5–2,5 g/l, bez obzira na sadržaj šećera, ne dolazi do intenzivne produkcije antifungalnih komponenti jer se u podlogama sa visokim sadržajem fosfata intenzivira rast biomase. Na osnovu pri-

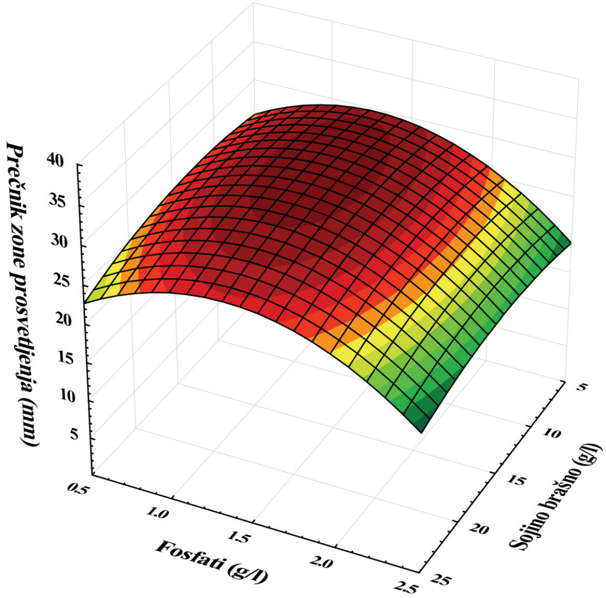


Slika 1. Uticaj početnog sadržaja glukoze i sojinog brašna na prečnik zone prosvetljenja prema Aspergillus spp. pri konstantnoj vrednosti sadržaja fosfata (1,5 g/l).
Figure 1. The effects of initial content of glucose and soybean meal on inhibition zone diameter for Aspergillus spp. at constant value of phosphates (1.5 g/l).



Slika 2. Uticaj početnog sadržaja glukoze i fosfata na prečnik zone prosvetljenja prema Aspergillus spp. pri konstantnoj vrednosti sadržaja sojinog brašna (15 g/l).
Figure 2. The effects of initial content of glucose and phosphates on inhibition zone diameter for Aspergillus spp. at constant value of soybean meal (15 g/l).

kazane odzivne površine uočava se da promena početnog sadržaja glukoze značajno utiče na biosintezu komponeti sa antifungalnim delovanjem ako je početni sadržaj fosfata u hranljivoj podlozi 0,5–1,5 g/l. Pri malim vrednostima sadržaja fosfata posmatrani odziv se intenzivira sa porastom sadržaja glukoze. Antagonistički efekat posmatranih faktora potvrđuje i negativna vrednost koeficijenta interakcije $b_{1,3}$ (tabela 2). Maksimalan prečnik zone prosvetljenja od oko 35 mm modelom je predviđen u opsegu početnog sadržaja šećera 45-50 g/l i fosfata 0,5–1,5 g/l.



Slika 3. Uticaj početnog sadržaja sojinog brašna i fosfata na prečnik zone prosvetljenja prema Aspergillus spp. pri konstantnoj vrednosti sadržaja glukoze (30 g/l).
Figure 3. The effects of initial content of soybean meal and phosphates on inhibition zone diameter for Aspergillus spp. at constant value of glucose (30 g/l).

Slika 3 prikazuje predikciju prečnika zone prosvetljenja prema *Aspergillus* spp. pri konstantnom sadržaju glukoze (30 g/l) i variranim sadržajima sojinog brašna i fosfata. Na osnovu prikazanih rezultata jasno se zapaža da je posmatrani odziv ujednačen u celokupnom ispitanom opsegu početnog sadržaja sojinog brašna, dok promena početnog sadržaja fosfata u manjoj meri utiče na sintezu komponenti antifungalnog dejstva. Dobijeni rezultati ukazuju da interakcija ova dva faktora, pri konstantnom sadržaju izvora ugljenika, nema veliki uticaj na sintezu antifungalnih agenasa. Maksimalan prečnik zone prosvetljenja od oko 30 mm prema predviđanjima modela je postignut pri početnom sadržaju sojinog brašna 5–15 g/l i fosfata 1,0–1,5 g/l.

Optimizacija sastava podloge

Krajnji cilj primene postupka odzivne površine je optimizacija željenog procesa tako da razvijeni modeli mogu biti korišćeni za simulaciju i optimizaciju. Za optimizaciju procesa sa dva ili više odziva najčešće se koristi koncept željene funkcije. Iako je ova metoda poznata više od dvadeset godina njena primena je bila ograničena usled nepostojanja softverskih paketa koji objedinjuju dizajn eksperimenta, regresionu analizu i optimizaciju. Program Design-Expert objedinjuje ove korake u opšti nelinearni algoritam. Generalni pristup sastoji se u konvertovanju individualnih odziva u individualne željene funkcije čije vrednosti se kreću od 0 do 1. Vrednost individualne željene funkcije „0“ predstavlja najlošiju vrednost, dok vrednost „1“ predstavlja najbolju vrednost posmatranog odziva. Ukupna željena funkcija jednaka je geometrijskoj sredini pojedinačnih željenih funkcija [25].

Za određivanje optimalnog početnog sadržaja glukoze, sojinog obezmašćenog brašna i fosfata u podlozi za proizvodnju antifungalnih komponenti primenjena je metoda željene funkcije. U zavisnosti od posmatranih odziva kao rezultat optimizacije dobijene su najveće moguće vrednosti ukupne željene funkcije sa ograničenjima definisanih minimizacijom ili maksimizacijom nekih od faktora i odziva, kao što je prikazano u tabeli 4.

Ako se kao jedini cilj optimizacije definiše postizanje najvećeg mogućeg prečnika zone prosvetljenja prema *Aspergillus* spp. (prvi set), koji je direktni pokazatelj količine sintetisanih antifungalnih komponenti, vrednost željene funkcije ima najveću vrednost (1,00) pri početnim sadržajima glukoze, sojinog brašna i fosfata od 47,77, 24,54 i 0,98 g/l, redom. Primenom hranljive podloge sa ovako definisanim početnim sadržajima nutrijenata model predviđa formiranje zone prosvetljenja prečnika 40,93 mm. Značajna je činjenica da je pri ovim uslovima predviđeni rezidualni sadržaj glukoze izuzetno visok i iznosi 19,36 g/l, a rezidualni sadržaj ukupnog azota 0,69 g/l.

Neiskorišćeni nutrijenti predstavljaju gubitke u ekonomskom smislu. Pored toga, otpadne vode ovakvog sastava nose značajno organsko opterećenje te stoga moraju da prođu zahtevan postupak obrade pre nego što se ispuste u životnu sredinu što poskupljuje proizvodni proces. Stoga su, pored postizanja najveće antifungalne aktivnosti kultivacione tečnosti, kao dodatne pojedinačne željene funkcije u drugom setu optimizacije zadate i minimizacija neiskorišćene glukoze i ukupnog azota. Pri ovim pojedinačnim željenim funkcijama, maksimalna vrednost ukupne željene funkcije iznosi 0,85 za početne sadržaje glukoze 24,11 g/l, sojinog brašna 5,00 g/l i fosfata 1,37 g/l. Pri navedenom sastavu hranljive podloge modelom predviđena zona prosvetljena, kao pokazatelj antifungalne aktivnosti, iznosi 32,26 mm, a rezidualni sadržaji glukoze i ukupnog azota 1,21 i 0,31 g/l, respektivno. Poređenjem rezultata prva dva seta optimizacije (tabela 4) zapaža se da se smanjenjem utroška glukoze za 49,53% i sojinog brašna za 79,62% i povećanjem utroška fosfata za 39,80% prečnik zone prosvetljenja smanji za 21,18%. Pri tome u kultivacionom medijumu zaostaju veoma male količine neutrošenih nutrijenata.

Cena sastojaka hranljive podloge učestvuje u ukupnim troškovima izvođenja biotehnološke proizvodnje sa značajnim udelom. Stoga su u trećem setu optimizacije kao uslovi, pored postizanja maksimalne zone prosvetljenja, definisani minimalan početni sadržaj glukoze, sojinog brašna i fosfata. Maksimalna vrednost ukupne željene funkcije od 0,78, ostvaruje se pri početnom sadržaju glukoze od 18,05 g/l, sojinog brašna od 5,00 g/l i fosfata od 0,87 g/l. Nakon biosinteze u kultivacionoj tečnosti, koja stvara zonu prosvetljenja prema *Aspergillus* spp. prečnika 27,72 mm, zaostaje sadržaj glukoze od 2,59 g/l i ukupnog azota od 0,28 g/l. Ovako definisani uslovi podrazumevaju manji utrošak glukoze za 25,13%, isti utrošak sojinog brašna i manji utrošak fosfata za 36,50% u odnosu na drugi set optimizacije

Tabela 4. Optimalne vrednosti ispitivanih parametara i predviđeni odzivi
Table 4. Optimized values of examined factors and predicted responses

Promenljive i odzivi	Prvi set		Drugi set	
	Zadata ograničenja	Procenjene vrednosti	Zadata ograničenja	Procenjene vrednosti
Glukoza, g/l	U opsegu	47,77	u opsegu	24,11
Sojino brašno, g/l	U opsegu	24,54	u opsegu	5,00
Fosfati, g/l	U opsegu	0,98	u opsegu	1,37
Prečnik zone prosvetljenja, mm	Max	40,93	max	32,26
Rezidualna glukoza, g/l	U opsegu	19,36	min	1,21
Rezidualni ukupan azot, g/l	U opsegu	0,69	min	0,31
Ukupna željena funkcija	1,00		0,85	
	Treći set		Četvrti set	
Glukoza, g/l	Min	18,05	min	13,56
Sojino brašno, g/l	Min	5,00	min	5,00
Fosfati, g/l	Min	0,87	min	1,02
Prečnik zone prosvetljenja, mm	Max	27,72	max	26,70
Rezidualna glukoza, g/l	U opsegu	2,59	min	1,08
Rezidualni ukupan azot, g/l	U opsegu	0,28	min	0,26
Ukupna željena funkcija	0,78		0,83	

dok je predviđeni prečnik zone prosvetljenja manji za 14,07%.

Sa ciljem definisanja sastava hranljive podloge pri kojem bi se ostvarila najveća ekonomičnost procesa proizvodnje komponenti sa antifungalnim delovanjem prema *Aspergillus* spp. postavljeni su sledeći uslovi: minimalan početni i rezidualni sadržaj ispitivanih nutrijenata uz ostvarenje najvećeg mogućeg prečnika zone prosvetljenja (četvrti set). Vrednosti variranih faktora, pri kojima je ovako definisana željena funkcija maksimalna (0,83), su početni sadržaj glukoze od 13,56 g/l, početni sadržaj sojinog brašna od 5,00 g/l i početni sadržaj fosfata od 1,02 g/l. Pri ovim uslovima, predviđene vrednosti odziva su sledeće: prečnik zone prosvetljenja od 26,70 mm, rezidualni sadržaj glukoze od 1,08 g/l i rezidualan sadržaj ukupnog azota od 0,26 g/l.

Dobijeni rezultati ukazuju da se ušteda sastojaka hranljive podloge može ostvariti limitiranjem izvora azota (sadržaj sojinog brašna u drugom, trećem i četvrtom setu iznosi 5 g/l). Na ovaj način se sprečava prekomerno umnožavanje biomase pa su stoga potrebe proizvodnog mikroorganizma u pogledu svih nutrijenata smanjene značajno više u odnosu na smanjenje prinosa antifungalnih agenasa, kao produkata sekundarnog metabolizma.

ZAKLJUČAK

Istraživanja iz okvira ovog rada ukazuju na veliki potencijal izolovanog soja *Streptomyces hygroscopicus* za proizvodnju antifungalnih agenasa koji se mogu primenjivati u biološkim metodama borbe protiv fitopatogenih gljiva iz roda *Aspergillus*. Primenjeni postupak optimizacije sastava hranljive podloge pokazao se kao efikasan, relativno jednostavan i, što je najvažnije, omogućava uštedu vremena i materijala koji su potrebni kod izvođenja velikog broja eksperimenata. Dobijeni rezultati predstavljaju osnovu za dalju tehnoekonomsku analizu procesa, koja bi razrešila opravdanost smanjenja količine produkovanih antifungalnih komponenti sa ciljem uštede sastojaka hranljive podloge kao i smanjenja organskog opterećenja otpadnih tokova.

Zahvalnica

Ovaj rad je rezultat istraživanja sprovedenih u okviru projekta pod brojem 114-451-3669/2012: “Optimizacija biotehnološkog postupka proizvodnje bioagenasa - antagonista skladišnih patogena jabuke“ finansiranog od strane Pokrajinskog sekretarijata za nauku i tehnološki razvoj Autonomne Pokrajine Vojvodine.

LITERATURA

[1] D. Nikolić, M. Fotirić, Oplemenjivanje jabuke u svetu, u Zborniku radova II Savetovanja "Inovacije u voćarstvu", Beograd, 2009, str. 5–22.

[2] R.J. McLaughlin, C.L. Wilson, S. Droby, R. Ben-Arie, E. Chalutz, Biological control of postharvest diseases of grape, peach, and apple with the yeasts *Kloeckera apiculata* and *Candida guilliermondii*, Plant Dis. **76** (1992) 470–473.

[3] R. Barkai-Golan, Species of *Aspergillus* causing postharvest fruit decay in Israel, Mycopathologia **71** (1980) 13–16.

[4] G. Perrone, A. Susca, G. Cozzi, K. Ehrlich, J. Varga, J.C. Frisvad, M. Meijer, P. Noonim, W. Mahakarnchanakul, R.A. Samson, Biodiversity of *Aspergillus* species in some important agricultural products, Stud. Mycol. **59** (2007) 53–66.

[5] R. Barkai-Golan, N. Paster, Mouldy fruits and vegetables as a source of mycotoxins: part 1, World Mycotoxin J. **1** (2008) 147–159.

[6] M. Grahovac, D. Inđić, S. Lazić, S. Vuković, Biofungicidi i mogućnosti primene u savremenoj poljoprivredi, Pestic. fitomed. **24** (2009) 245–258.

[7] Ž. Čeković, Zaštita bilja pomoću prirodnih pesticida, Hem. Ind. **60** (2006) 113–119.

[8] S. Khamna, A. Yokota, J.F. Peberdy, S. Lumyong, Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants, Int. J. Integr. Biol. **6** (2009) 143–147.

[9] [9] M. Oskay, Antifungal and antibacterial compounds from *Streptomyces* strains, Afr. J. Biotechnol. 8 (2009) 3007-3017.

[10] M. Thenmozhi, K. Kannabiran, Anti-*Aspergillus* activity of *Streptomyces* sp. VITSTK7 isolated from Bay of Bengal coast of Puducherry, India, J. Nat. Env. Sci. **2** (2011) 1–8.

[11] A. Ayari, H. Morakchi, K.G. Djamila, Identification and antifungal activity of *Streptomyces* sp. S72 isolated from Lake Oubeira sediments in North-East of Algeria, Afr. J. Biotechnol. **11** (2012) 305–311.

[12] H.M. Rifaat, N.H. Abd El Naser, S.M. Helmy, A.M. Ali, Taxonomical studies on certain streptomycetes exhibiting antimicrobial activity isolated from Egyptian soils, J. Cult. Collect. **5** (2006-2007) 25–34.

[13] M.A.M. Akhand, M.A.A. Al-Bari, M.A. Islam, P. Khondkar, Characterization and antimicrobial activities of a metabolite from new *Streptomyces* species from Bangladeshi soil, J. Sci. Res. **2** (2010) 178–185.

[14] M.M. Muharram, M.S. Abdelkader, S.I. Alqasoumi, Antimicrobial activity of soil actinomycetes isolated from Alkharj, KSA, Int. Res. J. Microbiol. **4** (2013) 12–20.

[15] N. Singh, V. Rai, Optimization of cultural parameters for antifungal and antibacterial metabolite from microbial isolate; *Streptomyces rimosus* MTCC 10792 from soil of Chhattisgarh, Int. J. Pharm. Pharm. Sci. **4** (2012) 94–101.

[16] A.A. Al-Askar, W.M. Abdul Khair, Y.M. Rashad, *In vitro* antifungal activity of *Streptomyces spororaveus* RDS28 against some phytopathogenic fungi, Afr. J. Agric. Res. **6** (2011) 2835–2842.

[17] M. Elibol, Optimization of medium composition for actinorhodin production by *Streptomyces coelicolor* A3(2) with response surface methodology, Process Biochem. **39** (2004) 1057–1062.

[18] N. Zhang, Z. Qi, H. Duan, Y. Xie, J. Yu, C. Lu, X. Liu, Optimization of medium composition for production of antifungal active substance from *Streptomyces hygroscopicus* BS-112, Afr. J. Microbiol. Res. **6** (2012) 71–80.

[19] X. Wang, L. Huang, Z. Kang, H. Buchenauer, X. Gao, Optimization of the fermentation process of actionmycete strain Hhs.015^T, J. Biomed. Biotechnol. **1** (2010) 1–10.

[20] S.T. Williams, M. Goodfellow, G. Alderson, E.M.H. Wellington, P.H.A. Sneath, M.J. Sackin, Numerical classification of *Streptomyces* and related genera, J. General Microbiol. **129** (1983) 1743–1813.

[21] T. Cross, in: S.T. Williams, M.E. Sharpe, J.G. Holt (Eds.), Bergey' s Manual of Systematic Bacteriology Vol. 4: Growth and Examination of Actinomycetes-Some Guidelines, Williams & Wilkins Company, Baltimore, MD, 1989, pp. 2340–2343.

[22] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. **31** (1959) 426–428.

[23] K. Herlich, Official Methods of Analysis of the Association of Official Analytical Chemists, 5th edn., Association of Official Analytical Chemists, Arlington, 1990, pp. 758–759.

[24] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Patho. **45** (1966) 493–496.

[25] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E.G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N.L. dos Santos, Box-Behnken design: An alternative for the optimization of analytical methods, Anal. Chim. Acta **597** (2007) 179–186.

SUMMARY

BIOSYNTHESIS OF COMPONENTS WITH ANTIFUNGAL ACTIVITY AGAINST *Aspergillus* spp. USING *Streptomyces hygroscopicus*

Jelena M. Dodić[1], Zorana Z. Rončević[1], Jovana A. Grahovac[1], Bojana Ž. Bajić[1], Olivera S. Korolija[2]

[1]*University of Novi Sad, Faculty of Technology, Novi Sad, Serbia*
[2]*Hemofarm a.d., Vršac, Serbia*

(Scientific paper)

Losses of apple fruit during storage are mainly caused by fungal phytopathogens. Traditionally, postharvest fungal disease is controlled by the application of synthetic fungicides. However, the harmful impact on environment as well as human health largely limits their application. To reduce these problems in agrochemicals usage, new compounds for plant protection, which are eco-friendly, should be developed. The aim of this study is optimization of medium composition in terms of glucose, soybean meal and phosphates content, by applying response surface methodology, for the production of agents with antifungal activity against *Aspergillus* spp. For biosynthesis was used strain of *Streptomyces hygroscopicus* isolated from the environment. Experiments were carried out in accordance with Box-Behnken design with three factors on three levels and three repetitions in the central point. Antifungal activity of the obtained cultivation mediums against *Aspergillus oryzae* and *Aspergillus niger* was determined, *in vitro*, using the diffusion – disc method. The desirability function was used for determination of optimal medium components. Achieved model predicts that the maximum inhibition zone diameter (40.93 mm) against test microorganisms is produced when the initial content of glucose, soybean meal and phosphates are 47.77, 24.54 and 0.98 g/l, respectively. To minimize the consumption of medium components and costs of effluents processing, additional three sets of optimization were made. The chosen method for optimization of medium components was efficient, relatively simple and time and material saving. Obtained results can be used for the further techno-economic analysis of the process to select optimal medium composition for industrial application.

Keywords: *Aspergillus* spp.• Antifungal compounds • *Streptomyces hygroscopicus* • Medium optimization

A modified random pore model for carbonation reaction of calcium oxide with carbon dioxide

Seyed Mohammad Mahdi Nouri, Habib Ale Ebrahim, Bahram NaserNejad

Chemical Engineering Department, Petrochemical Centre of Excellence, Amirkabir University of Technology, Tehran, Iran

Abstract

In this work, the random pore model was modified for a general concentration dependency and also bulk flow effect, in order to predict the carbonation reaction of calcium oxide with carbon dioxide. This reaction is one of the main methods for carbon dioxide capture from industrial flue gases. Different kinetic rate concentration functions were tested with the various literature experimental data for finding the best reaction constants and rate functions. Moreover, an exponential function for the diffusion of carbon dioxide through the product layer was proposed from the whole experimental conversion–time profiles.

Keywords: random pore model, calcium oxide, carbon dioxide, carbonation reaction.

Available online at the Journal website: http://www.ache.org.rs/HI/

SCIENTIFIC PAPER

UDC 66.095.6:54

Hem. Ind. **69** (2) 209–217 (2015)

doi: 10.2298/HEMIND131116011N

Global accumulation of CO_2 in the atmosphere has increased from 280 ppm in around 1860 to approximately 316 ppm in 1957 and rapidly to 390 ppm in 2010 [1]. Fossil fuels are the dominant form of energy utilized in the world (86%), and account for about 75% of the current anthropogenic CO_2 emission [2]. The need to reduce anthropogenic emissions of CO_2 is globally accepted and represents the driving force to reconsider the current technologies used for power generation [3].

The use of moderate temperature in the CO_2 separation step by lime has the potential to reduce efficiency penalties with respect to other methods. Within the temperature range of 400–800 °C, calcium oxide is the best choice among other metal oxides like potassium, lithium, sodium, and magnesium for reaction with CO_2. Also, the reversible carbonation/calcination reactions of calcium oxide have been proposed as the base of energy storage systems [4], and as a chemical heat pump [5]. Silaban *et al*. studied the reversibility of this reaction as the basis of a moderate temperature separation of CO_2 for the hydrogen purification [6].

A shrinking core model was applied by Johnsen *et al*. [7] to describe the carbonation reaction. Stendardo *et al*. used a modified grain model and the structural changes in the spherical grains are taken into account by the inclusion of a variable diffusivity of the gaseous reactant [8]. Bhatia and Perlmutter used a random pore model which considers the pore overlapping [9–12]. Sun et al. developed a discrete pore size distribution model with effective diffusivity in the product layer as the only fitting parameter [13]. Zero activation energy for surface reaction was obtained by Nitsch [14] and Bhatia and Perlmutter [12] and further supported by Dennis and Hayhurst [15]. Sun and his co-workers [16] obtained the activation energy of 29±4 kcal/mol for limestone and 24±6 kcal/mol for dolomite.

The reaction orders reported by different studies are not the same. Bhatia and Perlmutter claimed a first order reaction for CO_2 partial pressure of 0–10 kPa [12]. The experiments of Grasa et al. confirmed same results for up to 1 atm CO_2 partial pressure [17]. On the other hand, Kyaw *et al*. obtained a zero order for higher CO_2 partial pressures [18]. Sun et al. reported a first order for CO_2 partial pressures of less than 10 kPa, and a zero order for above this value [16].

The sudden transition of reaction rate is explained by the formation of a critical product layer by some researchers. Alvarez and Abanades estimated a product layer thickness of about 50 nm for the onset of slow reaction period [19]. They used a simple pore model and mercury porosimetry data to determine the critical product layer thickness. Barker reported a critical calcium carbonate layer thickness of 22 nm before diffusion control takes over in the progress of reaction [20]. Mess measured the product layer thickness of nonporous particles of the size range of 15–20 μm, and also a product layer thickness of 130 nm corresponding to the onset of the slow reaction period was reported [21].

In this work, carbonation reaction is investigated using a modified random pore model. The random pore model has shown good accuracy to predict some complicated gas-solid reactions such as the reaction of calcium oxide with sulphur dioxide which the solid structure changes during the reaction [22]. Data obtained by

---

Correspondence H.A. Ebrahim, Department of Chemical Engineering, Petrochemical Centre of Excellence, Amirkabir University of Technology, 424 Hafez Ave, Tehran, Iran.

E-mail: alebrm@aut.ac.ir

Paper received: 16 November, 2013

Paper accepted: 30 January, 2014

Bhatia and Perlmutter [12], Grasa *et al*. [17] and some experiments carried out by the authors have been used to calculate the kinetic constants and diffusivity coefficient. Due to the special characteristic of the carbonation reaction in which the reaction control mechanism changes very quickly, the random pore model is not sufficient to predict this reaction and some modifications should apply to this model. These improvements consist of a general concentration dependency, and also accounting for the bulk flow phenomenon. In all the previous works for modelling the carbonation reaction of lime with CO_2, the effect of bulk flow through the solid pellet was neglected. Since carbon dioxide is the only diffusing gas and there is not any product gas in the reaction, bulk flow effects should be considered for calculating the inherent kinetic constants. Moreover, the effect of sudden shift on the reaction rate was shown by changing product layer diffusivity in this work. Bhatia and Perlmutter [12] suggested two simplified equations for the kinetically controlled region and product layer diffusion control. However, in the present work, variable product layer diffusivity is used to predict the whole conversion-time profile with the fast changes in its slope.

EXPERIMENTAL PROCEDURE

The starting material used for the experiments was calcium carbonate (Merck Art No.: 102059) which was calcined in the TGA apparatus before the carbonation experiments. The flow diagram of the system for kinetic study of the $CaO+CO_2$ reaction is presented in Figure 1. This system consists of a thermogravimeter (TG) from Rheometric Scientific (model STA-1500).

High purity CO_2 and nitrogen were used for the experiments. The pellet is put on a platinum basket cell in the TG. The system is heated under an inert gas stream (gas 1) with the rate of 20 °C/min, up to the calcination temperature (900 °C). When the calcination reaction is finished (about 2 min), the temperature decreased to the isothermal carbonation reaction temperature. After 5 min, the system is switched to a reacting gas mixture (a predefined CO_2/N_2 mixture) as gas 2 and reaction begins at the desired temperature. At this condition, the weight changes are obtained versus time by TG at a constant temperature. The conversion was obtained from the following equation from the TG profiles:

$$X = \frac{\Delta w Mw_{\mathrm{CaO}}}{w_{\mathrm{i}} Mw_{\mathrm{CO_2}} \times \mathrm{Purity}} \tag{1}$$

The nitrogen adsorption and mercury porosimetry were used to evaluate the pore size distributions of the samples (pellets after calcinations and decomposition). Nitrogen adsorption (by Autosorb-1MP from Quantachrome) determines the porous structure in the range of micro and meso pores by a 55 points test. However, larger macro-pores that are out of the detection range of nitrogen adsorption can be determined by the mercury porosimetry. By using both of above methods and combining their results, the whole pore size distributions in the range of 0.3–10000 nm can be evaluated. The pore size distribution of calcined calcium carbonate is shown in figure 2.

Model description

Consider the following general gas–solid reaction:

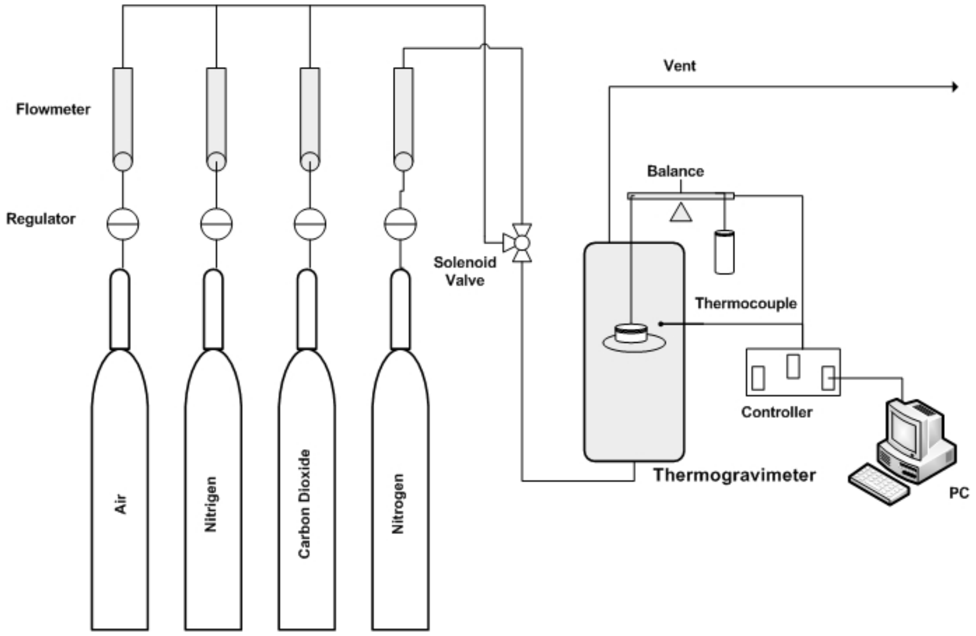


Figure 1. Experimental set up for the TG experiments.

$$\mathrm{A(g)} + \vartheta_{\mathrm{B}}\mathrm{B(s)} \rightarrow \vartheta_{\mathrm{C}}\mathrm{C(g)} + \vartheta_{\mathrm{D}}\mathrm{D(s)} \qquad (2)$$

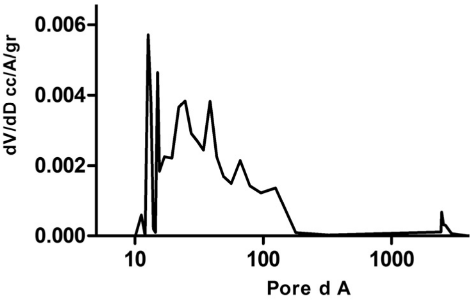


Figure 2. pore size distribution of calcined calcium carbonate.

The basic assumptions for formulating the random pore model are stated here:

(1) The reaction is initiated on the surfaces of pores in the solid B, and the nucleation of product D is rapid;

(2) The reactant B is isotropic and has no significant closed pore volume;

(3) The bulk gas concentration is constant;

(4) The pellet is isothermal;

(5) The pseudo-steady-state approximation for gas phase mass balance is valid;

(6) The pellet retains its original overall shape and size during the reaction.

The local reaction rate equation for the solid reactant is [11]:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{k_{\mathrm{S}} S_0 F(C)(1-X)\sqrt{1-\psi \ln(1-X)}}{\left(1-\varepsilon_0\right)\left[1+\frac{\beta Z}{\psi}\left(\sqrt{1-\psi \ln(1-X)}-1\right)\right]} \qquad (3)$$

where β is a modified Biot modulus. ψ is the random pore model parameter which is a function of initial pore size distribution of the pellet. Z is the ratio of molar volume of the solid product to the solid reactant as follows:

$$Z = \frac{\vartheta_{\mathrm{D}} \rho_{\mathrm{B}} M_{\mathrm{D}}}{\vartheta_{\mathrm{B}} \rho_{\mathrm{D}} M_{\mathrm{B}}} \qquad (4)$$

Using this equation, $Z = 2.17$ was obtained for the carbonation reaction of lime. The relation suggested by Wakao and Smith [23] is used to obtain the pore diffusion as a function of pellet porosity:

$$\delta = \frac{D_{\mathrm{e}}}{D_{\mathrm{e0}}} = \left(\frac{\varepsilon}{\varepsilon_0}\right)^2 = \left[1-\frac{(Z-1)(1-\varepsilon_0)(1-b)}{\varepsilon_0}\right]^2 \qquad (5)$$

For a pellet with spherical geometry, the mass balance for gaseous reactant A is:

$$\frac{d}{dr}\left(r^2 N_{\mathrm{A}}\right) = r^2 R_{\mathrm{A}} \qquad (6)$$

here R_{A} is the local rate of consumption of the fluid reactant A per unit volume. The molar flux, N_{A}, is given by:

$$N_{\mathrm{A}} = \left(N_{\mathrm{A}} + N_{\mathrm{C}}\right)x_{\mathrm{A}} - D_{\mathrm{e}} C_T \nabla x_{\mathrm{A}} \qquad (7)$$

And from the stoichiometry:

$$N_{\mathrm{A}} = -\vartheta_{\mathrm{C}} N_{\mathrm{C}} \qquad (8)$$

Substituting Eq. (8) in Eq. (7) and rearranging, we get:

$$N_{\mathrm{A}} = \frac{-D_{\mathrm{e}} C_T \mathrm{d}x_A / \mathrm{d}r}{1-(1-\vartheta_C)x_{\mathrm{A}}} \qquad (9)$$

Combining Eqs. (6) and (9), we obtain:

$$\frac{1}{r^2}\frac{\mathrm{d}}{\mathrm{d}r}\left(D_{\mathrm{e}} C_T r^2 \frac{\mathrm{d}x/\mathrm{d}r}{1-(1-\vartheta_{\mathrm{C}})x_{\mathrm{A}}}\right) - R_{\mathrm{A}} = 0 \qquad (10)$$

Equations (3) and (10) may be written in dimensionless form as:

$$\frac{1}{y^2}\frac{\partial}{\partial y}\left(\frac{\delta y^2}{1+\theta a}\frac{\partial a}{\partial y}\right) = \frac{\phi^2 f(a) b \sqrt{1-\psi \ln b}}{1+\frac{\beta Z}{\psi}\left(\sqrt{1-\psi \ln b}-1\right)} \qquad (11)$$

$$\frac{\partial b}{\partial \tau} = -\frac{f(a) b \sqrt{1-\psi \ln b}}{1+\frac{\beta Z}{\psi}\left(\sqrt{1-\psi \ln b}-1\right)} \qquad (12)$$

Equations (11) and (12) show the modification of random pore model when accounting for a general concentration dependency and bulk flow effect. The initial and boundary conditions are expressed as:

$$\begin{aligned} &\tau = 0 \rightarrow \quad b = 1 \\ &y = 0 \rightarrow \quad \frac{\partial a}{\partial y} = 0 \\ &y = 1 \rightarrow \quad \frac{1+\theta}{1+\theta a}\frac{\mathrm{d}a}{\mathrm{d}y} = Sh(1-a) \end{aligned} \qquad (13)$$

Because of the sudden sharp shift from the initially fast reaction to the slower reaction rate, the random pore model should be further modified in order to predict the carbonation reaction behaviour. In order to predict the abrupt change in the carbonation reaction rate, it is assumed that D_{p} is a function of solid conversion. This assumption was suggested by Stendardo *et al.* [8] for the carbonation reaction of lime:

$$D_{\mathrm{p}} = D_{\mathrm{P0}} \exp(-\alpha X^{\lambda}) \qquad (14)$$

The above equation is in agreement with findings of Mess *et al.* They reported that effective product layer diffusivity decreases with time and approaches a constant value for times greater than about 600 min [21].

The effective initial diffusivity of CO_2 through porous lime is obtained by:

$$\frac{1}{D_{e0}}=\frac{1}{\varepsilon_0^2}\left(\frac{1}{D_{AM}}+\frac{1}{D_{AK}}\right) \tag{15}$$

Where D_{AM} is the molecular diffusivity of CO_2 and is estimated from the equation presented by Slattery and Bird [24]. D_{Ak} is the Knudsen diffusivity and can be determined from the following equation for the pore model [25]:

$$D_{AK}=\frac{4\bar{r}}{6}\left(\frac{8R_gT}{\pi M_A}\right)^{\frac{1}{2}} \tag{16}$$

Finally, the relations for evaluating the random pore model parameters (such as ψ and S_0) from the initial pore size distribution of the solid reactant have been presented elsewhere [26].

RESULTS AND DISCUSSION

Figure 3 shows the conversion-time data at various temperatures and CO_2 concentrations obtained from TG for calcined $CaCO_3$ pellets. As can be seen in Figure 3, after an initial rapid growth, there is a sudden transition to a much slower reaction rate regime.

Figure 4 shows the effect of bulk flow on the solid conversion. It can be seen that the bulk flow effect increases with a progress of reaction and greater importance of product layer diffusion. It is noted that when the volume of the product gas is greater than the reactant gas ($\theta > 0$), the outward bulk flow makes it more difficult for the gaseous reactant to diffuse into the interior layer of the pellet. This results in a lower overall rate of reaction. When the volume of the product gas is smaller ($\theta < 0$), the inward bulk flow speeds up the diffusion, thus increasing the overall rate of reaction. For the carbonation reaction of calcium oxide with carbon dioxide there is not any product gas, and therefore, the bulk flow effect enhances the overall rate of the reaction.

In order to investigate the kinetics of the reaction at initial stages, it is assumed that the thickness of the product layer is quite thin, so diffusion through the product layer can be neglected. With this assumption, only intrapellet diffusion and intrinsic reaction kinetics are rate controlling. Thus, Eqs. (11) and (12) can be rearranged as:

$$\frac{1}{y^2}\frac{\partial}{\partial y}\left(\frac{\delta y^2}{1+\theta a}\frac{\partial a}{\partial y}\right)=-\frac{\partial b}{\partial \tau} \tag{17}$$

$$\frac{\partial b}{\partial \tau}=-f(a)b\sqrt{1-\psi \ln b} \tag{18}$$

The solid conversion can be computed from the solid concentration as:

$$X(\tau)=1-3\int_0^1 y^2 b(y,\tau)\mathrm{d}y \tag{19}$$

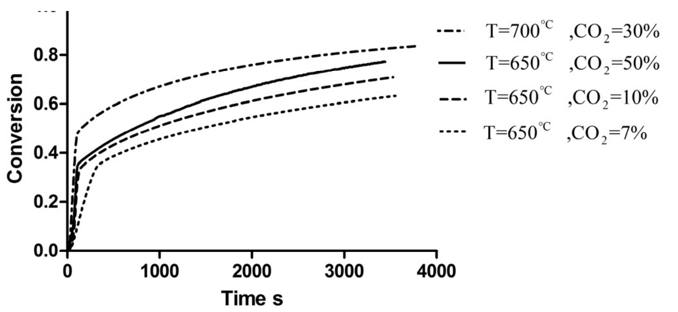


Figure 3. Conversion–time profiles of calcium oxide carbonation at different temperature and CO_2 partial pressures.

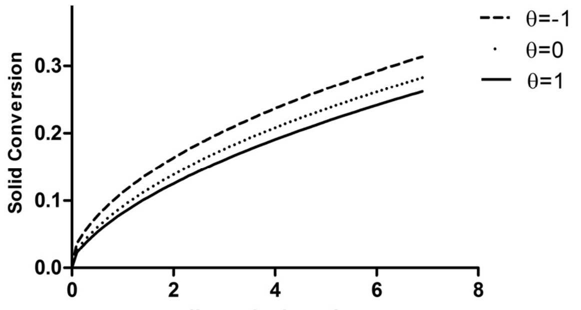


Figure 4. Effect of bulk flow on the solid conversion, β = 100, Φ = 10, ψ = 2.1.

The dimensionless solid concentration can be estimated by:

$$b = 1 - \int_0^1 f(a)\mathrm{d}\tau \qquad (20)$$

Differentiation of Eq. (19) yields:

$$\frac{\mathrm{d}X}{\mathrm{d}\tau} = 3\int_0^1 y^2 f(a)\mathrm{d}y \qquad (21)$$

which can be rearranged as:

$$\frac{(1-\varepsilon_0)}{S_0 3\int_0^1 y^2 f(a)\mathrm{d}y}\left[\frac{\mathrm{d}X}{\mathrm{d}t}\right]_{t\to 0} = k_s\left(C_A - C_{\mathrm{Aeq}}\right)^n \qquad (22)$$

$(\mathrm{d}X/\mathrm{d}t)_{t\to 0}$ is the initial slope of experimental conversion time data. By plotting the left hand side of equation (22) against C_{A} for each concentration dependency, k_S can be calculated. Moreover, the deviation of points from linearity shows the accuracy of the reaction rate functions f(a) for predicting the experimental data.

The CO_2 concentration driving force is limited by the equilibrium of the carbonation reaction which is reported by Baker as [20]:

$$\log p_{\mathrm{eq}} = 7.079 - \frac{8308}{T} \qquad (23)$$

This Eq. (23) shows the equilibrium pressures of about 0.07 and 0.12 atm at 700 and 800 °C, respectively. For flue gas concentration of coal combustion processes (12–15% of CO_2), the temperature above 850º$^{\mathrm{C}}$ is unfavourable for carbonation reaction.

The experimental data carried out by the TGA and the data of Bhatia and Perlmutter [12] and Grasa *et al.* [17] were used to determine the best order of reaction. Table 1 shows the correlation coefficients of each reaction order plot in Eq. (22).

As can be seen from Table 1, the fractional concentration dependency shows the best correlation. These results are in agreement with some other observations. Bhatia and Perlmutter [12] claimed a firsts order reaction for concentration of CO_2 between 0–10%. They obtained these results from an atmospheric thermogravimetric analyser. However, Sun et al. [16] and Kyaw *et al.* [18] observed a zero reaction order for higher CO_2 partial pressure. Therefore, the above proposed fractional reaction function will cover both results and change the reaction order from near one at low CO_2 partial pressures to near zero at higher CO_2 pressures. The low obtained activation energy is in agreement with the results of Bhatia [12] which did not observe any significant variation with changes in temperature. The same trend was also reported by Nitsch [14] at a higher temperature range (800–900 °C).

There is a sudden shift from the initial fast reaction rate to the slower second stage in the carbonation reaction of lime with CO_2. The data reported by some researchers show that the second stage reaction is independent of gas composition [21]. Thus, diffusion of carbon dioxide through the $CaCO_3$ product layer cannot be the rate controlling mechanism. This indicates that the reaction does not represent the simple simultaneous surface reaction and product layer diffusion process. Since the slow second stage rate is independent of CO_2 concentration, the diffusion mechanism must be in the solid phase if the rate is controlled by a product layer diffusion process.

The product layer diffusion function was evaluated from experimental data of the authors and Bhatia and Perlmutter [12], and kinetic constants were calculated. Product layer diffusion is assumed as an exponential function of solid conversion in Eq. (14) as an improvement in the random pore model.

The sudden shift in the carbonation reaction rate cannot be well predicted by the assumption of constant product layer diffusivity. Figure 5 shows a comparison between the experimental results of Bhatia and Perlmutter [12] and the model with a constant diffusivity. As can be seen in the figure, the model is not able to predict the experimental data successfully.

The values of parameters α and λ in the suggested function for D_{P} were obtained using trial and error at various operating temperatures. The comparison between the model predictions and the experimental conversion data for carbonation reaction is shown in

Table 1. Correlation coefficients of various reaction order functions

n	R^2		Expressions
	Bhatia's experiments	TG experiments	
1	0.994	0.885	–
2	0.954	0.735	–
0.5	0.990	0.782	–
$\frac{a}{1+k_{\mathrm{ad}}a}$	0.997	0.965	$k_s = 2.75\exp\left(\frac{7.132(\mathrm{kcal/mol})}{RT}\right)(\frac{\mathrm{cm}^4}{\mathrm{mols}})$ $k_{\mathrm{ad}} = 5000\ \frac{\mathrm{cm}^3}{\mathrm{mol}}$

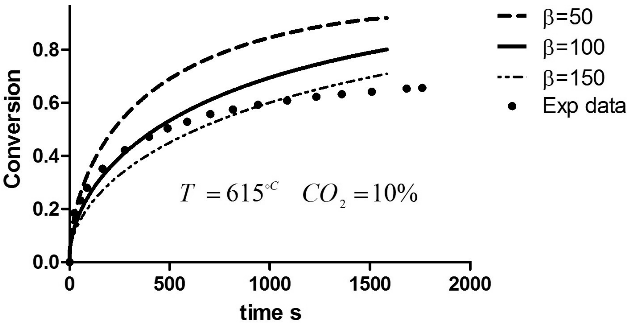


Figure 5. Comparison between the model predictions with constant product layer diffusivity and the experimental data of Bhatia and Perlmutter [12] at 615 °C and 10% CO_2.

Figures 6–8. As these Figures show, there is a very good agreement between model predictions and experimental data based on this variable product layer diffusion. Also from these figures, the product layer diffusion function is expressed as:

$$D_P = D_{P_0}\exp\left(-10X^4\right) \quad (24)$$

$$D_P \text{ at } X_f = 2.13\times10^{-7}\exp\left(-\frac{35.74\,\frac{\text{kcal}}{\text{mol}}}{RT}\right) \quad (25)$$

The model shows very good agreement with the data of Bhatia and Perlmutter [12] which can be seen in Figure 7. But for the TG experiment data and data of Grasa *et al.* [17], there is a small deviation between the experimental data and model results especially at the fast stage of reaction (Figures 6 and 8). Also, the transition between the fast stage of reaction and the slow stage for the data of Bhatia and Perlmutter [12] is not very sharp unlike the data of Garsa *et al.* [17] and our TG data (figure 6 and 8). It seems that the smooth form of carbonation reaction profiles in Bhatia and Perlmutter work [12] result in well prediction by the model. The macro pore texture of calcium oxide used by Bhatia seems to be responsible for his different TG profiles.

The low value of product layer diffusivity with a relatively high activation energy (35 kcal/mol), is quite a typical value associated with solid state diffusion. There are two suggested mechanisms for ion diffusion through the product layer:

A) Inward diffusion of CO_3^{2-} and outward diffusion of O^{2-} in order to maintain electroneutrality of the product layer.

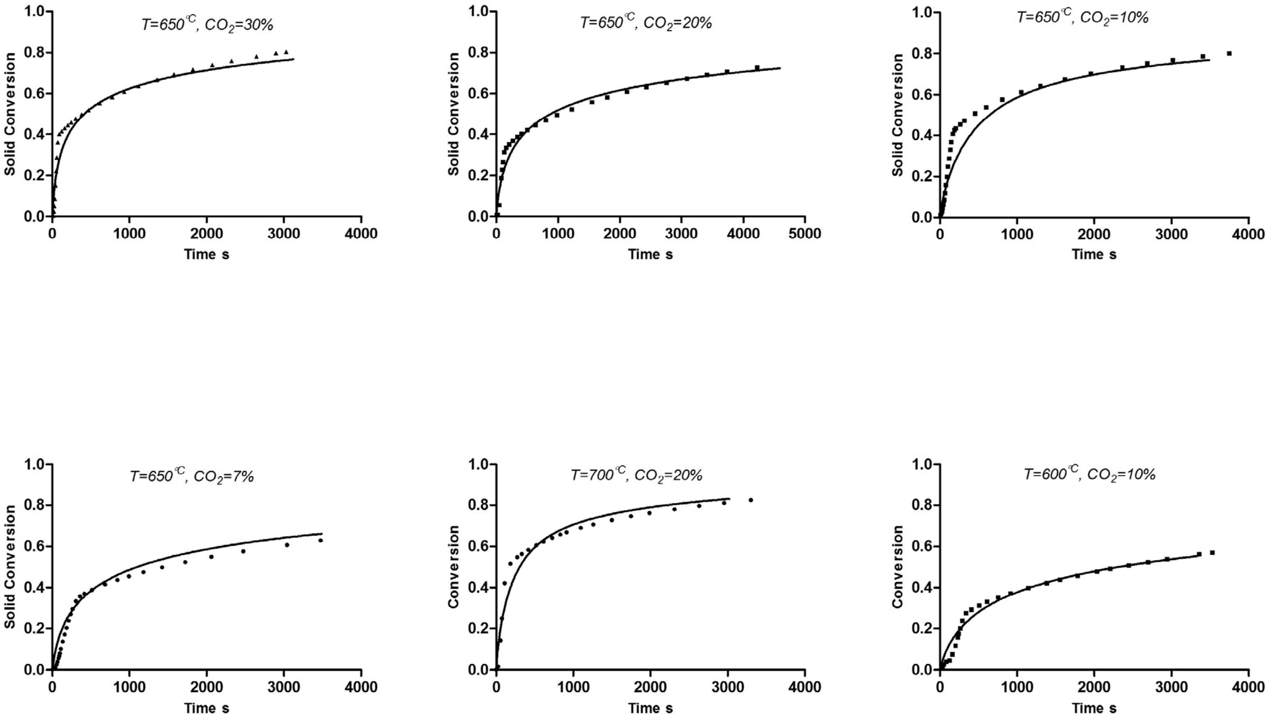


Figure 6. Comparison between the model predictions and the TG data at 650 °C and different CO_2 concentrations.

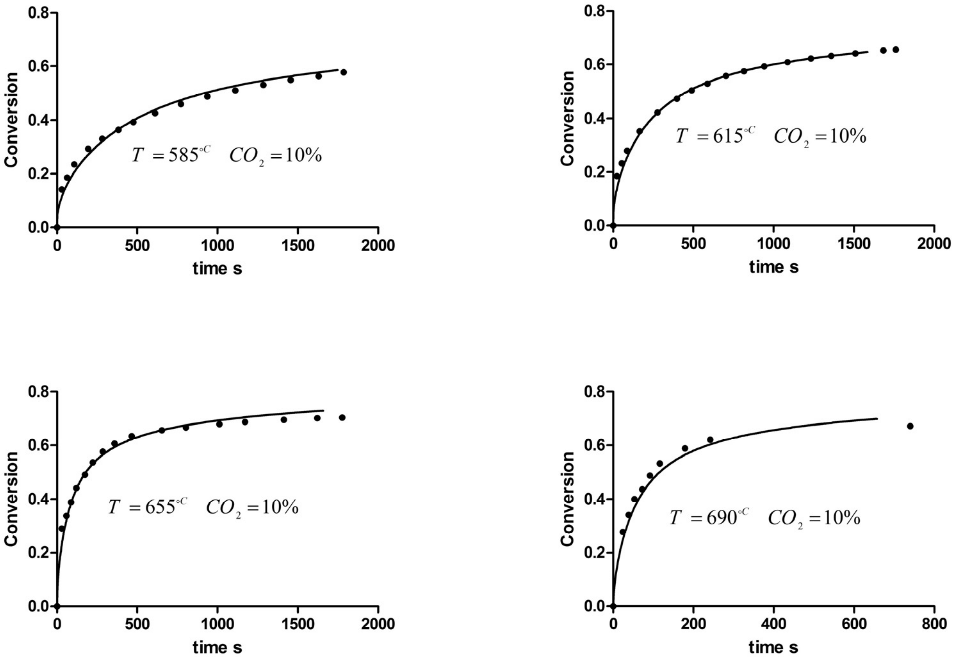


Figure 7. Comparison between improved random pore model predictions and experimental data of Bhatia and Perlmutter [12] at different temeratures.

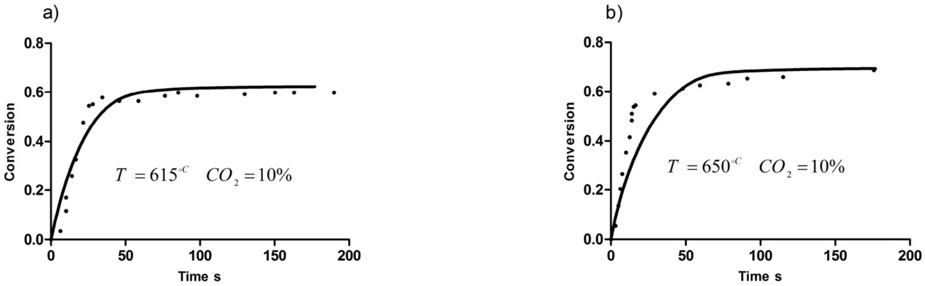


Figure 8. Comparsion between the model predictions and experimental data of Grasa et al. [17] at different temperatures.

B) Outward diffusion of Ca^{2+} and O^{2-} at the same time. Bhatia and Perlmutter suggested that the inward diffusion mechanism is dominant [12]. This idea is recently proven by Sun et al. who studied the dominating solid phase ionic transfer mechanism for diffusion of CO_2 through the $CaCO_3$ product layer [27].

Decomposition of CO_3^{2-} in the product layer can also be another diffusion mechanism. The CO_3^{2-} decomposes to a CO_2 molecule and O^{2-}. Then the CO_2 molecule moves to a vacant site in the neighbourhood. Similarly, another CO_2 molecule which decomposes elsewhere takes its place and composes the carbonate ion. By this mechanism, the CO_2 molecule moves site by site through the product layer. The possibility of this mechanism can be increased by consideration of value for decomposition heat of calcium carbonate (43.5 kcal/mol). It should be noted that there is not just one mechanism for the diffusion of CO_2 through the product layer and all of these phenomena may be involved simultaneously.

CONCLUSION

In this work, a modified random pore model for predicting the carbonation reaction of calcium oxide with carbon dioxide was developed. A general concentration rate function was considered in the modified mathematical model. Moreover, since there is not any product gas in the carbon dioxide reaction with calcium oxide, the bulk flow effect was accounted for in the random pore model. The Langmuir-Hinshelwood type rate function showed the best accuracy for the prediction of the experimental data. The kinetic constants for the reaction were calculated using this modified random pore model, and were compared with the TGA experimental data and the reported constants of Bhatia and Perlmutter [12] and Grasa *et al.* [17]. The diffusion of carbon dioxide through the product layer was also determined as an exponential function. It seems that the ionic solid phase mechanism for the product layer diffusion is dominant because of the low values for calculated diffusivities and their high activation energy.

Nomenclature

a	C_A / C_{Ab}, dimensionless gas concentration
b	C_B / C_{B0}, dimensionless solid concentration
C_A	gas concentration in the pellet (mol/cm^3)
C_{Ab}	bulk gas concentration (mol/cm^3)
C_B	solid reactant concentration (mol/cm^3)
C_{B0}	solid reactant concentration (mol/cm^3)
C_T	total gas concentration (mol/cm^3)
D_e	effective diffusivity of gas A in the pellet (cm^2/s)
D_{e0}	initial effective diffusivity of gas A in the pellet (cm^2/s)
D_P	effective diffusivity of gas A in the product layer (cm^2/s)
D_{P_0}	initial effective diffusivity of gas A in the product layer (cm^2/s)
k_m	external mass-transfer coefficient (cm^2/s)
k_s	surface rate constant ($cm^4/(mol\ s)$)
k_{ad}	adsorption coefficient
M_B	molecular weight of solid reactant (g/(g mol))
M_D	molecular weight of solid product (g/(g mol))
r	distance from the center of the pellet (cm)
R_0	radius of pellet (cm)
S_0	reaction surface area per unit volume (cm^2/cm^3)
Sh	Sherwood number for external mass transfer ($k_m R_0 / D_{e_0}$)
T	temperature (K)
t	time (s)
w	weight of solid (mg)
w_i	initial weight of solid reactant after calcination (calcium oxide) (mg)
Δw	weight gain of solid reactant during the carbonation reaction at each time t (mg)
x_i	mole fraction of species i
X	solid conversion at each time
y	dimensionless position in the pellet
Z	ratio of molar volume of solid product to solid reactant
ρ	product layer resistance, $2k_s\rho_B(1-\varepsilon_0)/M_B D_P S_0$
δ	variation ratio of the pore diffusion
ε	pellet porosity
ε_0	initial pellet porosity
θ	dimensionless bulk flow effect parameter, $(\vartheta_C - 1)x_{Ab}$
ϑ_i	stoichiometric coefficient of the reactant and product
ρ_B	true density of the solid reactant (g/cm^3)
ρ_D	true density of the solid product (g/cm^3)
τ	dimensionless time
ϕ	Thiele modulus for the pellet, $R_0\sqrt{k_s\rho_B S_0 / M_B D_{e_0}}$
ψ	random-pore model parameter.

REFERENCES

[1] S.D. Kenarsari, D. Yang, G. Jiang, S. Zhang, J. Wang, A.G. Russell, Q. Wei, M. Fan, Review of recent advances in carbon dioxide separation and capture, RSC Adv. **3** (2013) 22739–22773.

[2] J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. Van Der Linden, X. Dai, K. Maskell, C. Johnson, Climate change 2001: the scientific basis, Cambridge University Press, 2001.

[3] H.J. Herzog, What future for carbon capture and sequestration?, Environ. Sci. Technol. **35** (2001) 148A–153A.

[4] M. Aihara, T. Nagai, J. Matsushita, Y. Negishi, H. Ohya, Development of porous solid reactant for thermal-energy storage and temperature upgrade using carbonation/decarbonation reaction, Appl. Energ. **69** (2001) 225–238.

[5] Y. Kato, M. Yamada, T. Kanie, Y. Yoshizawa, Calcium oxide/carbon dioxide reactivity in a packed bed reactor of a chemical heat pump for high-temperature gas reactors, Nucl. Eng. Des. **210** (2001) 1–8.

[6] A. Silaban, M. Narcida, D.P. Harrison, Characteristics of the Reversible Reaction Between CO2(g) and Calcined Dolomite, Chem. Eng. Commun. 146 (1996) 149-162.

[7] K. Johnsen, J.R. Grace, S.S.E.H. Elnashaie, L. Kolbeinsen, D. Eriksen, Modeling of Sorption-Enhanced Steam Reforming in a Dual Fluidized Bubbling Bed Reactor, Ind. Eng. Chem. Res. **45** (2006) 4133–4144.

[8] S. Stendardo, P.U. Foscolo, Carbon dioxide capture with dolomite: A model for gas–solid reaction within the grains of a particulate sorbent, Chem. Eng. Sci. **64** (2009) 2343–2352.

[9] M. Avrami, Kinetics of Phase Change. II Transformation-Time Relations for Random Distribution of Nuclei J. Chem. Phys **8** (1940) 212–225.

[10] S.K. Bhatia, D.D. Perlmutter, A random pore model for fluid-solid reactions: I. Isothermal, kinetic control, AIChE J. **26** (1980) 379–386.

[11] S.K. Bhatia, D.D. Perlmutter, A random pore model for fluid-solid reactions: II. Diffusion and transport effects, AIChE J. **27** (1981) 247–254.

[12] S.K. Bhatia, D.D. Perlmutter, Effect of the product layer on the kinetics of the CO2-lime reaction, AIChE J. **29** (1983) 79–86.

[13] P. Sun, J.R. Grace, C.J. Lim, E.J. Anthony, A discrete-pore-size-distribution-based gas–solid model and its application to the reaction, Chem. Eng. Sci. **63** (2008) 57–70.

[14] W. Nitsch, Über die Druckabhängigkeit der $CaCO_3$-Bildung aus dem Oxyd, Z. Elektrochem. **66** (1962) 703–708.

[15] J.S. Dennis, A.N. Hayhurst, the effect of CO_2 on the kinetics and extent of calcination of limestone and dolomite particles in fluidised beds, Chem. Eng. Sci. **42** (1987) 2361–2372.

[16] P. Sun, J.R. Grace, C.J. Lim, E.J. Anthony, Determination of intrinsic rate constants of the CaO–CO_2 reaction, Chem. Eng. Sci. **63** (2008) 47–56.

[17] G. Grasa, R. Murillo, M. Alonso, J.C. Abanades, Application of the random pore model to the carbonation cyclic reaction, AIChE J. **55** (2009) 1246–1255.

[18] K. Kyaw, M. Kanamori, H. Matsuda, M. Hasatani, Study of Carbonation Reactions of Ca–Mg Oxides for High Temperature Energy Storage and Heat Transformation, J. Chem. Eng. Jpn. **29** (1996) 112–118.

[19] D. Alvarez, J.C. Abanades, Determination of the critical product layer thickness in the reaction of CaO with CO_2, Ind. Eng. Chem. Res. **44** (2005) 5608–5615.

[20] R. Barker, The reversibility of the reaction $CaCO_3 \rightleftarrows CaO+CO_2$, J. Appl. Chem. Biotechnol. **23** (1973) 733–742.

[21] D. Mess, A.F. Sarofim, J.P. Longwell, Product Layer Diffusion during the Reaction of Calcium Oxide with Carbon Dioxide, Energ. Fuels **13** (1999) 999–1005.

[22] S.K. Bhatia, D.D. Perlmutter, The effect of pore structure on fluid-solid reactions: Application to the SO_2-lime reaction, AIChE J. **27** (1981) 226–234.

[23] N. Wakao, J.M. Smith, Diffusion in catalyst pellets, Chem. Eng. Sci. **17** (1962) 825–834.

[24] J.C. Slattery, R.B. Bird, Calculation of the diffusion coefficient of dilute gases and of the self-diffusion coefficient of dense gases, AIChE J. **4** (1958) 137–142.

[25] J. Szekely, J.W. Evans, H.Y. Sohn, Gas-solid reactions, Academic Press, New York, 1976.

[26] H. Ale Ebrahim, Application of Random-Pore Model to SO_2 Capture by Lime, Ind. Eng. Chem. Res. **49** (2009) 117–122.

[27] Z. Sun, S. Luo, P. Qi, L.-S. Fan, Ionic diffusion through Calcite ($CaCO_3$) layer during the reaction of CaO and CO_2, Chem. Eng. Sci. **81** (2012) 164–168.

IZVOD

MODIFIKOVANI MODEL NASUMIČNIH PORA ZA REAKCIJU KARBONACIJE KALCIJUM-OKSIDA UGLJEN-DIOKSIDOM

Seyed Mohammad Mahdi Nouri, Habib Ale Ebrahim, Bahram NaserNejad

Chemical Engineering Department, Petrochemical Centre of Excellence, Amirkabir University of Technology, Tehran, Iran

(Naučni rad)

U ovom radu, model nasumičnih pora je modifikovan uvođenjem funkcije zavisnosti koncentracije i uzimanjem u obzir efekta toka, da bi se predvidela reakcija karbonacije kalcijum-oksida ugljen-dioksidom. Ova reakcija je jedan od glavnih metoda za hvatanje ugljen-dioksida iz industrijskih gasova. Testirane su različite funkcije zavisnosti koncentracije, na razne literaturne eksperimentalne podatke, da bi se dobila najoptimalnija funkcija i njene konstante. Takođe, na osnovu eksperimentalno utvrđene zavisnosti konverzije od vremena reakcije predložena je eksponencijalna funkcija za koeficijent difuzije ugljen-dioksida kroz sloj proizvoda.

Ključne reči: Model nasumičnih pora • Kalcijum-oksid • Ugljen-dioksid • Reakcija karbonacije