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# *Acta agriculturae Slovenica*

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Številka 1

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## NAČRTOVANJE IN IZGRADNJA RELACIJSKE PODATKOVNE BAZE ZA FUNKCIONALNA ŽIVILA\*

Simona JUVAN<sup>a)</sup>, Tomaž BARTOL<sup>b)</sup> in Bojana BOH<sup>c)</sup>

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### IZVLEČEK

Predstavljena je zasnova in izgradnja relacijske podatkovne baze za funkcionalna živila. To so živila, ki so del vsakodnevne prehrane in pozitivno vplivajo na zdravje ter zmanjšujejo tveganje za nastanek nekaterih bolezni. Relacijska podatkovna baza, zasnovana po Fienkelsteinovi metodi, vsebuje entitete, atribute in primarne ključe. Glavne entitete so: biološko aktivne snovi, klasifikacija, lastnosti, živila, fiziološki učinki, bolezni, postopki, zakonodaja, bibliografija in funkcionalna klasifikacija. Normalizirana baza, zgrajena v programu MS Access 2000, temelji na podatkovnem slovarju entitet, atributih, relacijah in entitetnem diagramu. Vsebuje podatke za 35 biološko aktivnih snovi, pridobljene iz 140 primarnih virov (članki, knjige ipd.). Klasifikacijsko drevo temelji na podlagi deskriptorjev iz tezavra FSTA, pridobljenih s pomočjo bibliometrične analize. Predstavljena je s šestimi med seboj povezanimi obrazci: Klasifikacija, Biološko aktivne snovi, Živilo, Bolezni, Zakonodaja, Bibliografija in Iskanje ter podaja odgovore na vprašanja: (1) kaj so funkcionalna živila, (2) katere aktivne snovi vsebujejo, (3) kako delimo biološko aktivne snovi (klasifikacija), (4) katere biološko aktivne snovi znižujejo tveganje za nastanek določenih bolezni, (5) kakšne so fizikalno kemijske lastnosti aktivnih snovi in (6) kakšna je zakonodaja na tem področju. Uporabniška aplikacija je dostopna kot CD-ROM in je namenjena širšemu krogu uporabnikov.

Ključne besede: funkcionalna živila / relacijske podatkovne baze / bibliografske podatkovne zbirke / načrtovanje / zbiranje podatkov / obdelava podatkov / klasifikacija / znanstvena informatika / bibliometrija / scientometrija / prehrana ljudi / zdrava hrana / preprečevanje bolezni / biološko aktivne snovi

\* V prispevku je povzet del rezultatov iz magistrskega dela z naslovom 'Bibliometrična analiza kot podpora za gradnjo relacijske podatkovne baze na področju funkcionalnih živil', ki jo je avtorica izdelala na Naravoslovnotehniški fakulteti pod mentorstvom prof. dr. B. Boh in somentorskim vodstvom doc.dr. T. Bartola

## DESIGN AND DEVELOPMENT OF A RELATIONAL DATABASE FOR FUNCTIONAL FOODS\*

### ABSTRACT

We present design and construction of a relational database for functional foods which are defined as foods used in everyday diet, and having beneficial effects on health as well as reducing risks for diseases. Database was designed according to Fienkelstein. It contains entities, attributes, and primary keys. Main entities are represented by: biologically active compounds, classification, properties, foods, physiological effects, diseases, processes, legislation, bibliography, and functional classification. Database was normalised and was constructed with MS Access 2000. It is based on dictionary of entities, attributes, relations, and entity-relationship diagram. It contains data for 35 biologically active foods. Classification tree was set up on the basis of descriptors from FSTA thesaurus following a bibliometric analysis. The data were extracted from 140 documents (articles, books etc.). The database is represented with six inter-linked forms (sheets): Classification, Biologically active compounds, Foods, Diseases, Legislation, Bibliography and Retrieval (Search). It is possible to search according to the following queries: (1) what are functional foods, (2) which active compounds are contained in foods, (3) classification of compounds, (4) which are the risk-reducing compounds, (5) physico-chemical properties of compounds, and (6) legislation in the field of functional foods. Application is available as CD-ROM and is accessible to end-users.

**Key words:** functional foods / relational databases / bibliographic databases / planning / design / data collection / data processing / classification / documentation / information science / bibliometrics / scientometrics / human nutrition / health foods / disease prevention / biologically active compounds

### UVOD

Hiter razvoj informacijske tehnologije omogoča učinkovito povzemanje in pretvorbo pomembnih informacij iz ogromne količine podatkov v uporabno obliko (Roth in sod., 2002). To se kaže predvsem na področju svetovnega spletja. Število specializiranih podatkovnih baz in informacijskih sistemov se povečuje na vseh področjih. Zmožnost organiziranja znanstvenih in industrijskih informacij v znanje vpliva na kvaliteto in napredek znanstvenih raziskav, industrijskega razvoja in izobraževanja (Boh, 1996). Podatkovne baze omogočajo enostaven dostop do rezultatov poskusov vključno z ustrezno literaturo ter s tem zmanjšujejo podvajanje raziskav (Nelson in sod., 2003). Razvoj informacijske tehnologije ponuja nova orodja za gradnjo relacijskih podatkovnih baz, vendar pa nam ta orodja niso v pomoč, če so podatkovne baze slabo načrtovane.

Tudi na področju živilstva obstaja nekaj teoretičnih opisov razvoja specializiranih podatkovnih baz. Wise (1994) je zbral podatke o dietetičnih izdelkih različnih proizvajalcev in ugotovil velike razlike v vsebnosti hranil v teh izdelkih. V ta namen je izdelal relacijsko podatkovno bazo, ki naj bi pospešila razvoj standardov na tem področju. Ovaskainen in sod. (1996) so prikazali, do kakšnih problemov lahko pride pri gradnji podatkovnih baz, ki podajajo podatke o vsebnosti hranil v živilih. Poudarili so, da je potrebno jasno določiti, na kakšen način pridobimo podatke za gradnjo baze. Pennington (2002) je podal pregled podatkovnih baz, ki predstavljajo vsebnost biološko aktivnih snovi v živilih in predlagal razvoj obširnejše in specializirane podatkovne baze za biološko aktivne snovi, ki bi vsebovala tudi opise živil ter podrobnejše analitične rezultate iz posameznih študij.

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\* The article presents a part of results from master thesis 'Bibliometric analysis as support for relational database design for functional foods', carried out by the autor at Natural Sciences and Engineering Faculty under supervision of prof. B. Boh, Ph.D. and cosupervision of T. Bartol, Ph.D.

Poleg tovrstnih podatkovnih baz obstajajo tudi podatkovne baze, ki omogočajo spremljanje in nadzor postopkov v živilski industriji. Primer takšne podatkovne baze za področje predelave mleka so predstavili Fitzgerald in sod. (1998). Le ta omogoča spremljanje kvalitete kontrole in posledično izboljšanje proizvodnje. Northrop in Cowell (1997) ter Swihart (2000) so predstavili podatkovni bazi iz pivovarstva, ki omogočata izboljšanje postopkov v pivovarski industriji, medtem ko je Weaver (2000) podal primer uporabe podatkovne baze na področju priprave in dostave hrane (*catering*), ki naj omogoča povečanje učinkovitosti te dejavnosti.

Del metodologije naše raziskave zajema tudi področje bibliometrije oz. scientometrije, ki pa je obsežno samostojno podpodročje znotraj informacijske znanosti, zato podajamo le bibliografski podatek o novejšem domačem viru, ki navaja pregled drugih izbranih virov s področja biotehniške scientometrije (Bartol, 2002).

V delu je predstavljena zasnova in izgradnja relacijske podatkovne baze za funkcionalna živila. Živilo je spoznano za funkcionalno, če je dokazan učinek, ki pozitivno vpliva na eno ali več funkcij v telesu, na način, da ohranja zdravje oziroma zmanjšuje možnosti za nastanek bolezni (Roberfroid, 2000). S pomočjo bibliometrične analize ključnih besed dokumentov s področja funkcionalnih živil v podatkovni zbirki FSTA smo določili glavna področja raziskovanja na raziskovalnem področju funkcionalnih živil, ki so služila kot entitete pri načrtovanju relacijske podatkovne baze. Zgrajena relacijska podatkovna baza podaja pregled nad funkcionalnimi lastnostmi živil in izpostavi, katere biološko aktivne snovi pozitivno vplivajo na zdravje oziroma zmanjšujejo tveganje za nastanek nekaterih bolezni. Uporabnikom je dostopna na CD-ROM-u.

## MATERIAL IN METODE DELA

FSTA (Food Science and Technology Abstracts) je mednarodna bibliografska podatkovna zbirka, ki pokriva področje živilskih znanosti in tehnologije ter raziskove s področja prehrane ljudi. Zapisi v zbirki vsebujejo bibliografske informacije, vsebinsko sintetizirane po načelu indeksiranja z deskriptorji in v večini primerov opremljene z izvlečkom. Zbirka se gradi od leta 1969 dalje in se redno dopolnjuje z novimi zapisi.

Na področju kmetijstva obstaja kar nekaj objav o relacijskih podatkovnih bazah. Pregledali smo število objavljenih dokumentov o relacijskih podatkovnih bazah na širšem področju kmetijstva v treh pomembnejših kmetijskih zbirkah AGRIS (AGRicultural Information System), CAB (Commonwealth Agricultural Bureaux Abstracts) in FSTA (Food Science and Technology Abstracts).

Dokumente smo pridobili z naslednjo iskalno sintakso:

“*relational database\**” or “*relational data base\**”

in

*relational and (database\* or data base\*)*.

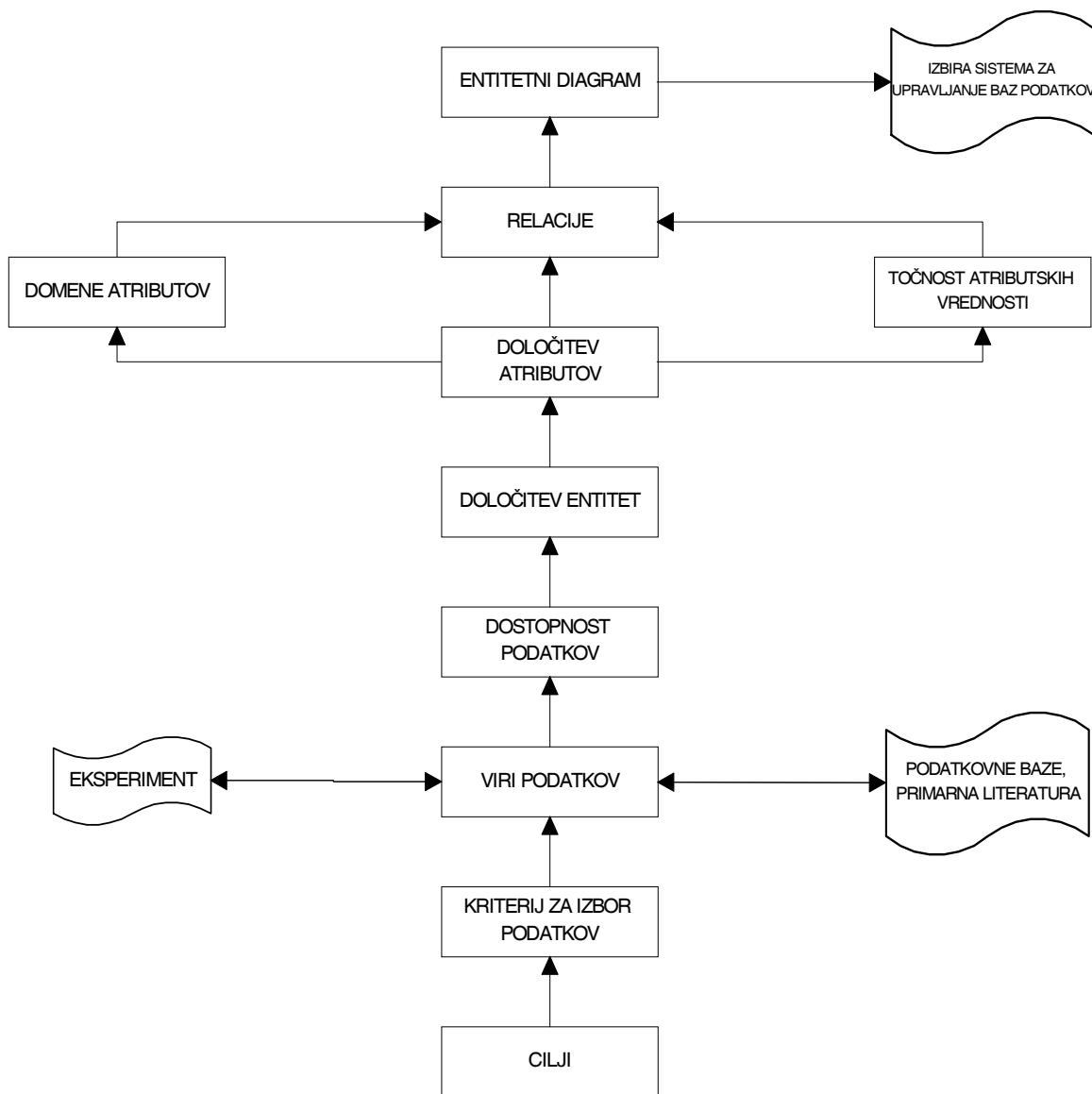
Izdelavo relacijske baze oziroma informacijskega sistema smo izvedli po naslednjih stopnjah (Vrtačnik in sod., 1992; Glažar in Vrtačnik, 2001):

- Določanje skupine strokovnjakov, ki sodelujejo pri izgradnji.
- Postavljanje jasnih ciljev, ki naj jih doseže izgradnja relacijske baze.
- Postavljanje vprašanj, na katere bo mogoče odgovoriti s pomočjo relacijske baze.
- Določanje kriterijev za izbor potrebnih podatkov in ugotovljanje, ali ti podatki že obstajajo in kako priti do njih.
- Določanje entitet in atributov.
- Izdelava entitetnega diagrama.
- Izbira ustreznega sistema za upravljanje baz podatkov in izgradnjo relacijske podatkovne baze.

Za postopek entitetno-relacijskega modeliranja smo uporabili metodologijo po Finkelsteinu (1989), ki temelji na izdelavi entiteno-relacijskih modelov. Elementi modela so entiteta, atribut in relacija. Metoda zajema naslednje faze:

- Definicija glavnih entitet, primarnih in zunanjih ključev.
- Določanje neključnih atributov.
- Normalizacija.
- Izdelava podatkovnega slovarja.
- Izdelava entitetnega diagrama.

Relacijsko podatkovno bazo smo nato zgradili s pomočjo programskega orodja MS Access 2000.



Slika 1. Metodologija pri gradnji relacijske podatkovne baze (Vrtačnik in Dolničar, 1996).

Figure 1. Methodology and steps in the construction of relational databases (Vrtačnik in Dolničar, 1996).

Ključne besede, pridobljene z bibliometrično analizo zadetkov za funkcionalna živila in biološko aktivne snovi v bazi FSTA (slika 3 in 4), smo v drevesasto strukturo razporedili po metodi strukturiranja podatkov v sisteme (Kornhauser, 1982).

## REZULTATI Z RAZPRAVO

### **Poizvedba v mednarodnih podatkovnih zbirkah s področja kmetijstva**

Iz Preglednice 1 je razvidno število objavljenih dokumentov o relacijskih podatkovnih bazah, ki se nahajajo v posameznih podatkovnih zbirkah s področja kmetijstva, od leta 1990 dalje.

Preglednica 1. Število dokumentov o relacijskih podatkovnih bazah v podatkovnih zbirkah AGRIS, CAB in FSTA, pridobljenih z dvema podobnima iskalnima sintaksama (poizvedba dne 21.4.2005)

Table 1. Occurrence of records with regard to the topic of relational databases in the databases AGRIS, CAB and FSTA retrieved with two similar search queries (searches from 21.4.2005)

Podatkovna zbirka	“relational database*” <i>OR</i> “relational data base*”	relational and (database* <i>OR</i> data base*)
AGRIS (1990–)	46	54
CAB (1990–)	276	316
FSTA (1990–)	14	14

Za ugotovitev natančnega števila dokumentov, ki govorijo le o relacijskih podatkovnih bazah s področja živilstva, bi bilo potrebno ročno pregledati vse dobljene zapise. Podrobnejše iskalno geslo, npr. (*nutri\** *or* *food\**) *and* relational and (database\* *or* data base\*) ni priklicalo vseh relevantnih dokumentov. Če dokument govorí samo o relacijskih bazah na področju nekega specifičnega živila, namreč sploh ni nujno, da ta dokument oz. bibliografski zapis vsebuje splošne prehranske izraze, kot sta npr. *food* ali *nutrition*.

### **Zasnova relacijske baze podatkov za funkcionalna živila**

Določitev skupine strokovnjakov, ki sodelujejo pri izgradnji

Pri gradnji relacijske podatkovne baze so sodelovali strokovnjaki s področja živilstva in informatike.

Postavitev jasnih ciljev, ki jih bo izpolnjevala relacijska baza

Cilj relacijske podatkovne baze je podati pregled funkcionalnih lastnosti živil in izpostaviti, katere biološko aktivne snovi pozitivno vplivajo na zdravje, oziroma zmanjšujejo tveganje za nastanek nekaterih bolezni.

Opredelitev vprašanj, na katere bo relacijska baza nudila odgovore

Relacijska podatkovna baza naj daje odgovore na naslednja vprašanja:

- Katera so funkcionalna živila.
- Katere biološko aktivne snovi vsebujejo funkcionalna živila.
- Kako delimo biološko aktivne snovi – klasifikacija.
- Katere biološko aktivne snovi zmanjšujejo tveganje za nastanek posameznih bolezni.
- Kakšne so fizikalno kemijske lastnosti biološko aktivnih snovi.

- Kakšni so fiziološki učinki posameznih biološko aktivnih snovi.
- Kakšna je zakonodaja na področju funkcionalnih živil.

Določitev kriterijev za izbor podatkov ter opredelitev podatkovnih virov

Podatke za gradnjo relacijske podatkovne baze smo dobili z analizo primarne literature (članki, prispevki na konferencah, knjige ...). Uporabili smo 140 virov, ki so bili dostopni v primarni obliki preko različnih ponudnikov elektronskih časopisov ter v knjižnicah Biotehniške fakultete.

Opredelitev entitet in atributov

Z že prej omenjeno bibliometrično analizo, ki je predstavljala ločen segment raziskave, smo opredelili devet glavnih področij raziskovanja. Bibliometrična analiza je upoštevala ključne besede iz dokumentov s področja funkcionalnih živil, kot jih zajema podatkovna zbirka FSTA. Tako smo pridobili devet glavnih entitet. Te predstavljajo *biološko aktivne snovi, klasifikacija, lastnosti, živila, fiziološki učinki, bolezni, postopki, zakonodaja in bibliografija*.

Kot dodatno entiteto smo vključili tudi *funkcionalno klasifikacijo*, saj se v večini virov biološko aktivne snovi delijo glede na funkcionalnost, npr. na *prebiotike, antioksidante, probiotike* ipd.

Glavne entitete so predstavljene v preglednici 2.

Preglednica 2. Opis glavnih entitet

Table 2. Description of primary entities

Ime glavne entitete	Opis
BAS	Ime biološko aktivnih snovi, ki pozitivno vplivajo na zdravje oziroma zmanjšujejo tveganje za nastanek določene bolezni, podatek o posledicah pomanjkanja in posledicah presežka ter priporočen dnevni vnos biološko aktivnih snovi.
Klasifikacija	Klasifikacija glede na sestavo (npr. mikroorganizmi, organske snovi, anorganske snovi).
Funkcionalna klasifikacija	Klasifikacija glede na to, v katero funkcionalno skupino spada biološko aktivna snov (npr. prebiotiki, fitoestrogeni, fitosteroli).
Lastnost	Opis fizikalno-kemijskih in senzoričnih lastnosti biološko aktivnih snovi.
Živilo	Opis živil, v katerih se nahajajo biološko aktivne snovi in količina le teh v živilu.
Fiziološki učinek	Opis fizioloških učinkov, ki jih povzroči posamezna biološko aktivna snov.
Bolezen	Opis bolezni in podatki, katere biološko aktivne snovi zmanjšujejo možnost za nastanek bolezni.
Postopek	Opis postopkov, s katerimi lahko povečamo koncentracijo biološko aktivnih snovi v živilih.
Zakonodaja	Opis zakonodaje na področju funkcionalnih živil v različnih državah.
Bibliografija	Opis virov, ki opisujejo posamezne entitete.

Sledil je postopek določevanja *primarnih ključev, zunanjih ključev in neključnih atributov* (preglednica 3). Primarni in zunanji ključi opredeljujejo medsebojno povezano entitet. Neključni atributi pa povedo, kakšne lastnosti ima posamezna entiteta. Sledil je postopek normalizacije, kjer smo povezave s pomočjo dodatne entitete *mnogo:mnogo (m:n)* pretvorili v povezavo *ena:mnogo (1:m)*.

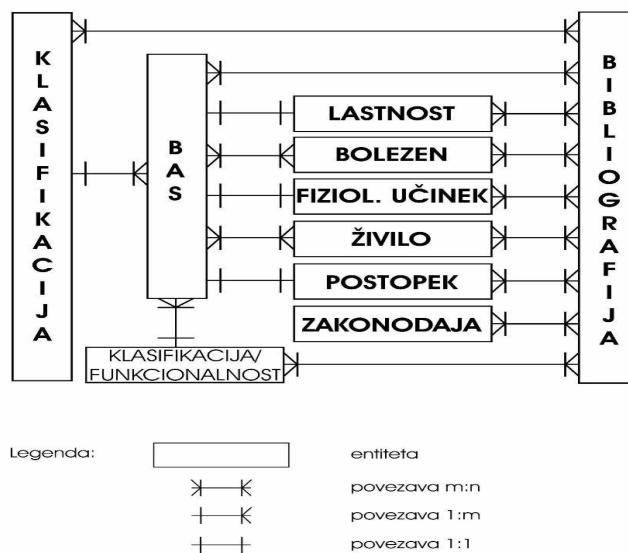
Preglednica 3. Entitete s primarnimi in zunanjimi ključi ter neključnimi atributi po normalizaciji

Table 3. Entities with the primary and external keys and non-key attributes after normalisation

Entiteta	Primarni in zunanji ključi, neključni atributi
BAS	ID-bas#, ime bas, dnevni vnos, posledice pomanjkanja, posledica presežka, ID-klasifikacija#, ID-funkcionalna klasifikacija#
Klasifikacija	ID-klasifikacija#, skupina, podskupina1, podskupina2, podskupina3, podskupina4
Funkcionalna klasifikacija	ID-funkcionalna klasifikacija#, funkcionalna skupina
Lastnost	ID-lastnost#, sinonim, številka CAS, kemijska formula, strukturna formula, tališče, vrelišče, gostota, barva, vonj, okus, tekstura, ID-bas#
Fiziološki učinek	ID-fiziološki učinek#, vloga bas v telesu, funkcija bas, ID-bas#
Živilo	ID-živilo#, skupina živila
Bas-živilo	ID-bas#, ID-živilo#, količina bas
Bolezen	ID-bolezen#, ime bolezni, opis bolezni
Bas-bolezen	ID-bas#, ID-bolezen#, vpliv bas na bolezen
Postopek	ID-postopek#, ime postopka, opis postopka za povečanje bas v živilu, ID-bas#
Zakonodaja	ID-zakonodaje#, država, ime zakona, opis zakona
Bibliografija	ID-bibliografije#, avtor, naslov, vir, letnik, številka, stran, leto, tip dokumenta
Bas-bibliografija	ID-bas#, ID-bibliografija#
Živilo-bibliografija	ID-živilo#, ID-bibliografija#
Fiziološki učinek-bibliografija	ID-fiziološki učinek#, ID-bibliografija#
Bolezen-bibliografija	ID-bolezen#, ID-bibliografija#
Postopek-bibliografija	ID-postopek#, ID-bibliografija#
Zakonodaja-Bibliografija	ID-zakonodaja#, ID-bibliografija#

### Izdelava entitetnega diagrama

Entitetni diagram (slika 2) grafično prikazuje povezavo med posameznimi entitetami.

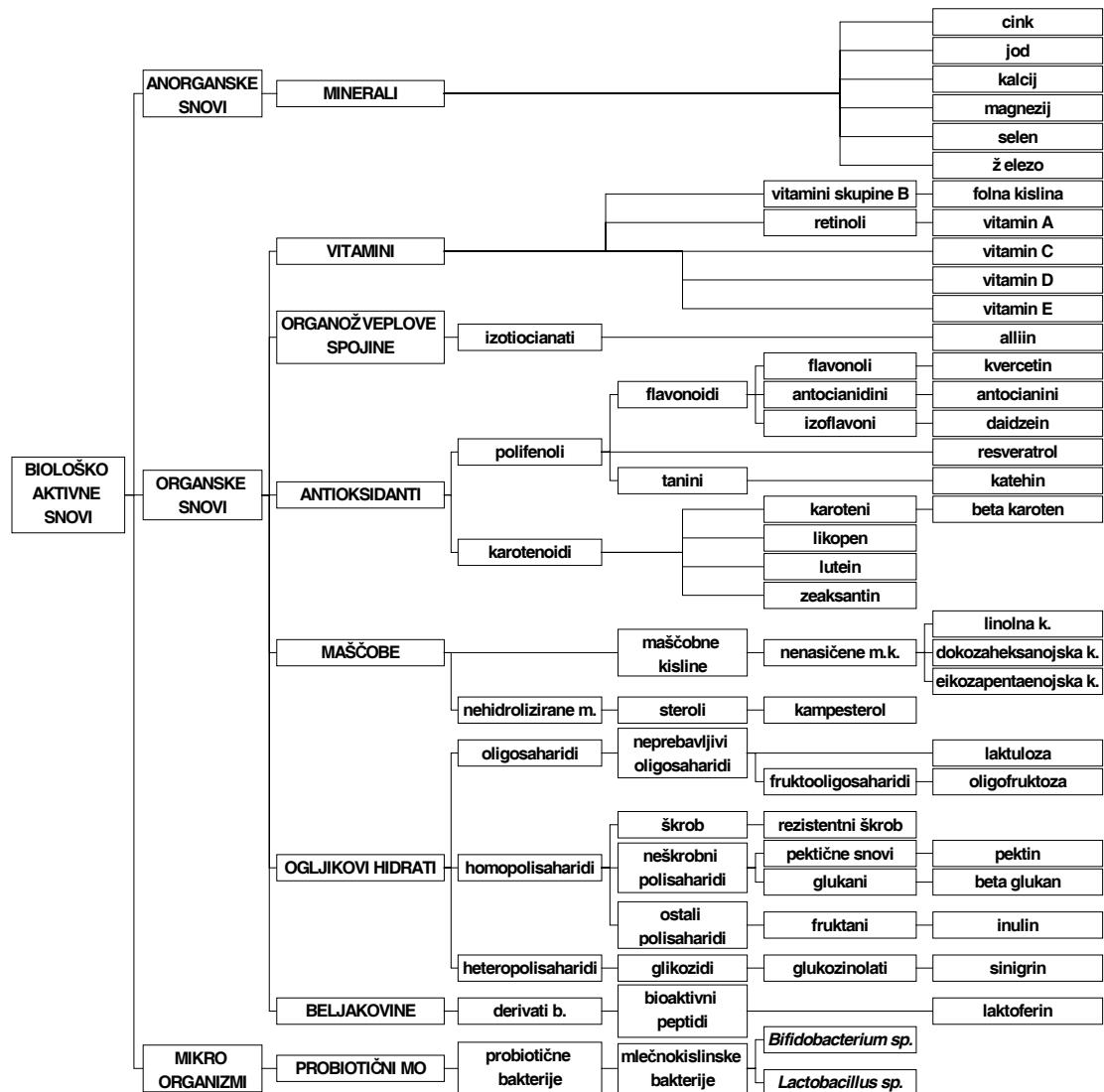


Slika 2. Entitetni relacijski diagram za relacijsko bazo funkcionalnih živil.

Figure 2. Relational diagram of entities in the relational database for functional foods.

### Izbor ustreznega sistema za izgradnjo in upravljanje relacijske podatkovne baze

Relacijsko podatkovno bazo za funkcionalna živila smo zgradili s pomočjo programskega orodja MS Access 2000. Entitete modela smo prenesli v tabele, attribute pa v stolpce. Posamezna vrstica predstavlja podatke o posamezni biološko aktivni snovi.



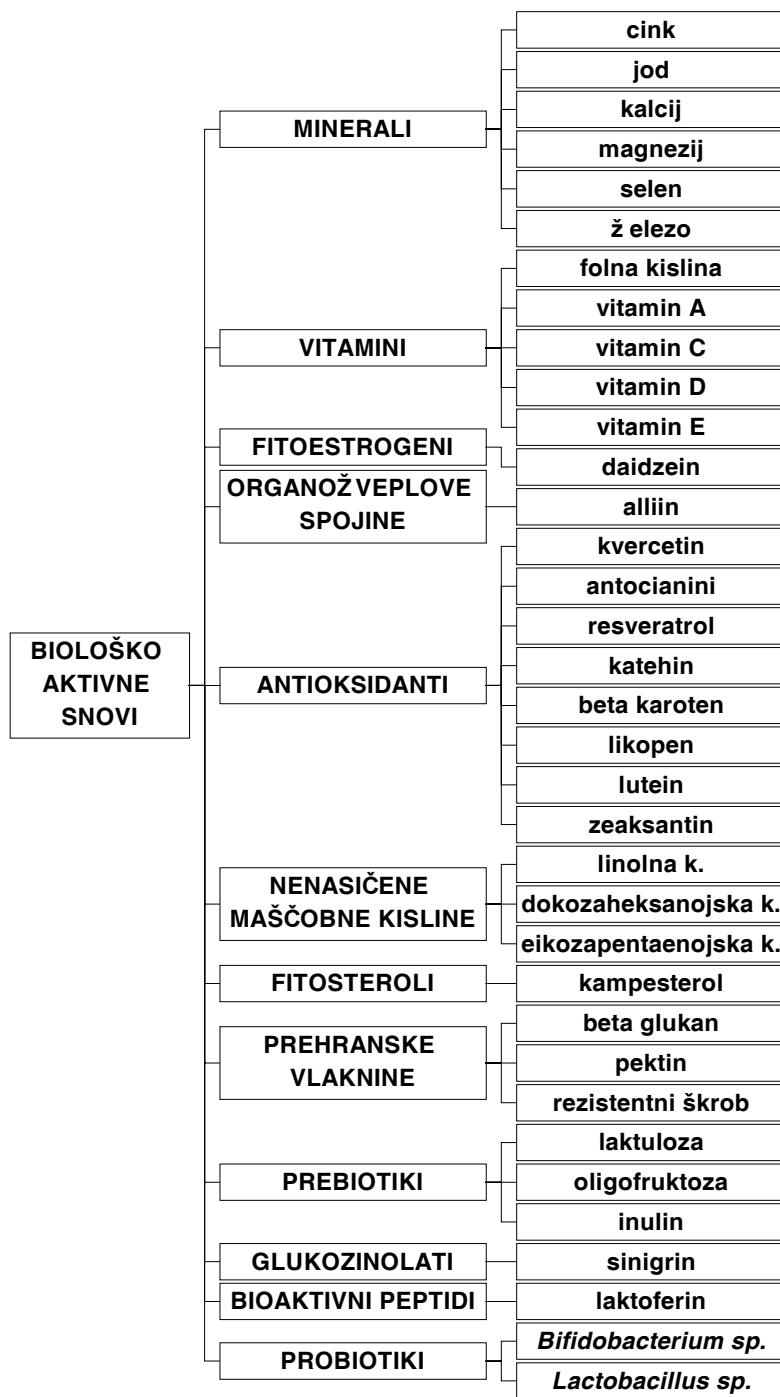
Slika 3. Delitev biološko aktivnih snovi uporabljenih v relacijski podatkovni bazi glede na sestavo.

Figure 3. Arrangement of biologically active components employed in the database with regard to the composition.

Bazo sestavlja devet osnovnih tabel:

- BAS (biološko aktivna snov)
- Klasifikacija
- Klasifikacija – funkcionalnost
- Lastnost
- Fiziološki učinek
- Živilo

- Bolezen
- Zakonodaja
- Bibliografija



Slika 4. Delitev biološko aktivnih snovi, uporabljenih v relacijski podatkovni bazi, glede na funkcionalnost.

Figure 4. Chemical arrangement of biologically active components employed in the database with regard to functionality.

Najprej smo v MS Access vnesli osnovne tabele (osnovne entitete) in njihove vrednosti (attribute).

Zbrali, uredili in vnesli smo podatke za 35 biološko aktivnih snovi, ki smo jih pridobili iz 140 virov oz. primarnih dokumentov, kot so npr. članki ali knjige. Biološko aktivne snovi, ki smo jih vključili v relacijsko podatkovno bazo, smo izbrali torej na podlagi lastne vsebinske analize teh dokumentov.

Slika 3 prikazuje delitev biološko aktivnih snovi, uporabljenih v bazi, glede na sestavo, medtem ko slika 4 prikazuje delitev biološko aktivnih snovi glede na funkcionalnost. Obe drevesasti strukturi vključujeta ključne besede, ki smo jih pridobili z bibliometrično analizo zadetkov za funkcionalna živila v bazi FSTA, nato pa smo jih hierarhično uredili po načelu od splošnega (levo) proti specifičnemu (desno) z metodo strukturiranja podatkov v sisteme.

### **Uporaba relacijske baze podatkov za funkcionalna živila**

Relacijsko podatkovno bazo za funkcionalna živila smo tehnično uredili tako, da je uporabniško dostopna tudi na CD-ROM-u. Na slikah 5–8 so predstavljeni meniji iz uporabniške aplikacije. Glavni meni relacijske podatkovne baze (slika 5) predvideva šest obrazcev: *Klasifikacija*, *Biološko aktivne snovi*, *Živilo*, *Bolezni*, *Zakonodaja*, *Bibliografija* in *Iskanje*.



Slika 5. Naslovna relacijske podatkovne baze za funkcionalna živila.

Figure 5. Title page of the user application of relational database for functional foods.

V obrazcu *Klasifikacija* (slika 6) so mogoče povezave do posameznih funkcionalnih skupin biološko aktivnih snovi. Če odpremo npr. obrazec *Minerali*, se pokažejo biološko aktivne snovi, ki spadajo v to funkcionalno skupino (slika 7). V tem obrazcu so shranjeni podatki o tem, v katero skupino glede na sestavo in v katero funkcionalno skupino sodi določena biološko aktivna snov, priporočen dnevni vnos, posledice pomanjkanja in presežka, vloga v telesu ter funkcija. Obrazec *Klasifikacija* omogoča povezavo do obrazec *Podrobnejša delitev BAS (funkcionalnost)* in *Podrobnejša delitev BAS (kemijsko)*, kjer je predstavljena podrobnejša delitev biološko aktivnih snovi, bodisi po načelu funkcionalnosti bodisi po načelu kemijske zgradbe.



Slika 6. Obrazec Klasifikacija.

Figure 6. Form (Sheet) Classification.

<b>BIOLOŠKO AKTIVNA SNOV</b>	
Ime BAS	cink
FUNKCIONALNA SKUPINA	minerali
Ime skupine	Anorganske komponente
Ime podskupine 1	Minerali
Ime podskupine 2	BREZ
Dnevni vnos	15-19 mg
Posledice pomanjkanja	Zapozneta spolna zrelost, utrujenost, izguba okusa, zmanjšan apetit, daljše celjenje ran, zapoznlost v rasti, sterilnost.
Posledice presežka	Bruhanje.
Vloga v telesu	✓ krvi, možganih srcu, prostati, v vseh celicah.
Funkcija	Je kofaktor več kot 300 encimov. Sodeluje pri celjenju ran in opreklin. Potreben je za presnovno ogljikovih hidratov, za pravilno delovanje prostate, za rast in dozorevanje spolnih organov. Sodeluje pri metabolizmu vitamina B1, fosforja, beljakovin, maščob, nukleinskih kislin ter ostalih mikroelementov. Cink stabilizira molekulsko strukturo celičnih komponent in membran ter prispeva k vzdrževanju popolnosti celii in organov. Cink ima bistveno vlogo v prepisovanju polinukleotidov in v procesu genske ekspresije. Prav tako igra pomembno vlogo v imunskem sistemu. Cink se absorbira v tankem čревusu.

Slika 7. Obrazec Minerali.

Figure 7. Form (Sheet) Minerals.

Obrazec *Iskanje* (slika 8) omogoča iskanje po treh sklopih:

- **Biološko aktivna snov** (ime, posledice pomanjkanja, posledice presežka, funkcionalne skupine biološko aktivnih snovi)
- **Živilo** (ime živila, skupina živila)
- **Bolezzen** (ime bolezni, opis bolezni)

<b>ISKANJE PO RELACIJSKI PODATKOVNI BAZI</b>		<b>Zapri</b>
Biološko aktivna snov	<input type="text"/>	
Posledice pomanjkanja	<input type="text"/>	
Posledice presežka	<input type="text"/>	
Funkcionalna skupina	<input type="text"/>	<b>Iskanje po BAS</b>
Bolezni	<input type="text"/>	
Opis bolezni	<input type="text"/>	<b>Iskanje po bolezni</b>
Živilo	<input type="text"/>	
Skupina živila	<input type="text"/>	<b>Iskanje po živilu</b>

Slika 8. Obrazec Iskanje.

Figure 8. Form (Sheet) Search (Retrieval).

Uporabnik lahko relacijsko bazo uporablja po načelu zaslonskega pregledovanja (*browsing*) po posameznih med seboj povezanih sklopih (npr. klasifikacija, biološko aktivne snovi, živilo, bolezni, zakonodaja, bibliografija) ter po načelu standardnega iskanja s pomočjo besed oz. iskalnih izrazov (*searching*).

## SKLEPI

S pomočjo bibliometrične analize ključnih besed iz zapisov s področja funkcionalnih živil v podatkovni zbirki FSTA in s pomočjo celotnih primarnih dokumentov smo določili in oblikovali devet glavnih podpodročij raziskovanja funkcionalnih živil. Ta podpodročja smo uporabili kot glavne entitete pri zasnovi relacijske podatkovne baze. Dodali smo še entiteto *funkcionalna klasifikacija*, ki je pomembna pri opredeljevanju funkcionalnosti živil.

Relacijska podatkovna baza predstavlja pregled funkcionalnih lastnosti živil in biološko aktivnih snovi, ki pozitivno vplivajo na zdravje oziroma preprečujejo možnosti za nastanek bolezni. Uporabniki lahko s pomočjo te baze ob uporabi ustreznih ukazov poiščejo odgovore na naslednja vprašanja: (1) katera so funkcionalna živila, (2) katere biološko aktivne snovi vsebujejo živila, (3) kako delimo biološko aktivne snovi (klasifikacija), (4) katere biološko aktivne snovi znižujejo tveganje za nastanek določenih bolezni, (5) kakšne so fizikalno kemikske lastnosti biološko aktivnih snovi in (6) kakšna je zakonodaja na področju funkcionalnih živil. Podatkovna zbirka je namenjena tekočemu obveščanju uporabnikov o novostih in ugotovitvah na področju funkcionalnih živil, zato je predvideno sprotno ažuriranje podatkov.

Ciljni uporabniki izdelane relacijske baze, ki je dostopna na CD-ROM-u, so predvsem študentje in strokovnjaki s področja živilstva pa tudi vsi drugi, ki jih zanima ta problematika.

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## PREDICTION OF CHEMICAL COMPOSITION AND ENERGY VALUE OF HAY BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

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### ABSTRACT

One hundred and fifty-eight hay samples with known chemical composition and *in vitro* determined concentration of net energy for lactation (NEL) were scanned over the wavelength range from 1100 to 2500 nm at 8 nm intervals. Calibration equations for the prediction of dry matter (DM), crude protein (CP), crude fibre (CF), crude fat, ash and NEL were developed by the use of principal component analysis. NIRS demonstrated high predictive ability for CP ( $R^2 = 0.98$ ), CF ( $R^2 = 0.95$ ) and ash ( $R^2 = 0.94$ ). Only moderate accuracy was characteristic for DM (0.87) and crude fat ( $R^2 = 0.75$ ). With exception of ash deviations from reference methods are comparable to those which are expected by the use of the same reference methods in different laboratories. NIRS has a high ability to predict *in vitro* assessed NEL concentration ( $R^2 = 0.89$ ). More than 95% of samples lied within acceptable limits of  $\pm 0.3 \text{ MJ NEL kg}^{-1}$  dry matter (DM). Despite suboptimal sample distribution, i.e. small number of samples in classes below 4.4 and above 5.6  $\text{MJ NEL kg}^{-1}$  DM, the deviations of NIRS predicted values from reference values were not related to concentration of NEL. It was concluded that NIRS is rapid and reliable technique for determination of chemical composition and energy value of hay.

Key words: feed / hay/ net energy for lactation/ chemical composition/ NIRS

## NAPOVEDOVANJE KEMIČNE SESTAVE IN ENERGIJSKE VREDNOSTI MRVE Z BLIŽNJO INFRARDEČO REFLEKSIJSKO SPEKTROSKOPIO (NIRS)

### IZVLEČEK

Stooseminpetdesetim vzorcem mrve z znano kemično sestavo in *in vitro* določeno vsebnostjo neto energije za laktacijo (NEL) smo na vsakih 8 nm izmerili spektre reflektirane bližnje infrardeče svetlobe v valovnem območju med 1100 in 2500 nm. S pomočjo analize glavnih component smo razvili umeritvene enačbe za napovedovanje vsebnosti suhe snovi (SS), surovih beljakovin (SB), surove vlaknine (SVI), surovih maščob, pepela in NEL. Metoda NIRS je bila zelo dobra pri napovedovanju SB ( $R^2 = 0.98$ ), SVI ( $R^2 = 0.95$ ) in pepela ( $R^2 = 0.94$ ). Za surove maščobe in SS je bila značilna zmerna točnost (0,75 in 0,87). Z izjemo pepela so bila odstopanja od referenčnih metod primerljiva z odstopanjimi, ki jih lahko pričakujemo pri izvajaju istih referenčnih metod v različnih laboratorijih. Metoda NIRS je bila zelo dobra tudi pri napovedovanju *in vitro* ocenjene vsebnosti NEL ( $R^2 = 0.89$ ). Več kot 95 % vzorcev je ležalo znotraj sprememljivih meja  $\pm 0.3 \text{ MJ NEL kg}^{-1}$  sušine (SS). Kljub neoptimalni porazdelitvi vzorcev, t.j. majhnemu številu vzorcev v razredih pod 4,4 in nad 5,6  $\text{MJ NEL kg}^{-1}$  SS, odstopanja z NIRS ocenjenih vrednosti od referenčnih vrednosti niso bila povezana z vsebnostjo NEL. Sklenili smo, da je NIRS hitra in zanesljiva metoda za ocenjevanje kemične sestave in energijske vrednosti mrve.

Ključne besede: krma / mrva/ neto energija za laktacijo/ kemična sestava/ NIRS

## INTRODUCTION

The nutritive value of forages can be estimated on the basis of *in vivo* or *in vitro* digestibility trials or on the basis of chemical composition and digestibility coefficients from tables. All these methods have several drawbacks. *In vivo* digestibility methods can not be used in every-day praxis because experimental animals and large quantities of forages are needed. Besides, they are laborious, time-consuming and costly. The time and quantity of forage required for determination of digestibility can be reduced by introduction of *in vitro* methods (Tilley and Terry, 1963; Menke *et al.*, 1979). However, the problem of experimental animals remains. For a long time, nutritive value has been estimated on the basis of chemical composition and digestibility coefficients from tables. The main deficiency of this method is inaccuracy. Both, systematic error due to inadequacy of tables as well as unexplained deviations from *in vivo* or *in vitro* assessed NEL concentration were observed in samples of fresh forages, hays and silages (Babnik and Verbič, 2000; Babnik *et al.*, 2001, 2002). There is a substantial need for fast, accurate and simple methods for the estimation of nutritive value of forages. In recent years, the use of near infrared reflectance spectroscopy (NIRS) has expanded dramatically. To relate NIRS optical measurements to the chemical composition or nutritive value of forages the process of calibration has to be carried out. The aim of the present study was to examine the reliability of NIRS to predict chemical composition and energy value of hay.

## MATERIAL AND METHODS

### **Chemical composition and estimation of net energy for lactation (NEL)**

One hundred and fifty-eight hay samples were collected during the period of four years. All samples were dried in a ventilated oven at 60 °C and ground with the Brabender mill to pass 1 mm screen. Analyses of moisture, crude protein, crude fibre, crude ash and crude fat were done according to the methods described by Naumann and Bassler (1976). Samples were also tested with the *in vitro* Hohenheim gas test (Menke *et al.*, 1979) using the modified method as described by Blümmel and Ørskov (1993). About 200 mg of sample was exactly weighed in graduated 100 ml glass syringes. Thirty ml of inoculum (rumen liquor and artificial anaerobic saliva) was added to each syringe and incubated in water bath at 39 °C for 24 hours. Gas volume for each sample was measured in triplicates. Deviations caused by rumen liquor activity were corrected according to the standard hay sample which was provided by the University of Hohenheim. Net energy for lactation (NEL, in MJ kg<sup>-1</sup> DM) was calculated on the basis of corrected gas volume produced in 24 hours (GV<sub>24</sub>), crude fat and crude fibre (CF) (both in g kg<sup>-1</sup> DM) using the regression equation proposed by Aiple *et al.* (1995) (equation [1]).

$$\text{NEL} = 2.88 + 0.0754 \times \text{GV}_{24} + 0.000398 \times \text{crude fat} \times \text{crude fat} - 0.00340 \times \text{CF} \quad [1]$$

### **Near-infrared reflectance spectroscopy (NIRS)**

Samples were scanned in full rectangular cups with NIRSystems 6500 Monochromator (Foss NIRSystem, Silver Spring, MD). The system operated with software equipment (Win ISI – version 1.50) in the region from 1100 to 2500 nm at 8 nm intervals to give 173 data points for each sample spectrum. Data points were recorded as absorbance values (log (1/R); where R means reflectance). Principal component analysis (PCA) method was used to reduce spectral data to independent sources of variation and converted to scores to replace the spectra. At the same time samples with atypical spectral characteristics were eliminated using the CENTER

algorithm which eliminates samples with the Mahalanobis distance (GH) bigger than 3.0 from the mean of the file. Samples with Mahalanobis distance (NH) smaller than 0.6 between neighbours, which are considered to add no spectral variation to the population, were also eliminated. SELECT algorithm was used for this purpose.

## RESULTS AND DISCUSSION

### Chemical composition and concentration of net energy for lactation

Chemical composition and energy concentration in forage samples is presented in Table 1. A wide range in chemical composition and NEL concentration was covered and it can be considered that samples covered the range typical for samples from Slovenian farms.

Table 1. Chemical composition ( $\text{g kg}^{-1}$  DM) and concentration of net energy for lactation (NEL,  $\text{MJ kg}^{-1}$  DM) in samples of hay ( $n = 158$ )

Preglednica 1. Kemična sestava ( $\text{g kg}^{-1}$  SS) in koncentracija neto energije za laktacijo (NEL,  $\text{MJ kg}^{-1}$  SS) v vzorcih mrve ( $n = 158$ )

	Mean $\pm$ SE Sredina $\pm$ SE	Minimum Najmanj	Maximum Največ
Dry matter Suha snov	966 $\pm$ 14	916	983
Crude protein Surove beljakovine	111 $\pm$ 28	56	200
Crude fibre Surova vlaknina	312 $\pm$ 37	216	390
Crude fat Surove maščobe	20.9 $\pm$ 6.4	6.1	36.5
Ash Pepel	78.0 $\pm$ 19.2	30.6	154.8
NEL	4.97 $\pm$ 0.41	3.87	5.98

SE – standard error/ standardna napaka; NEL – net energy for lactation/ neto energija za laktacijo

### Accuracy of calibration equations

Determination coefficients ( $R^2$ ), standard errors of calibration (SEC) and standard errors of cross validation (SECV) of calibration equations are presented in Table 2. The highest accuracy of calibration equations was achieved for CP, CF and ash which have the highest  $R^2$  (0.94–0.98) and relatively low SEC and SECV values. Similar parameters of calibration equations for CP and CF were reported in the literature (Norris *et al.*, 1976; Herrero *et al.*, 1996; Bruno-Soares *et al.*, 1998; Park *et al.*, 1998; Lavrenčič *et al.*, 2001). In agreement with previous work (Berarado *et al.*, 1997; Bruno-Soares *et al.*, 1998; Park *et al.*, 1998) are also results for ash, although, somewhat lower  $R^2$  were also reported (De Boever *et al.*, 1996; Lavrenčič *et al.*, 2001). The dry matter determination coefficient ( $R^2$ ) was slightly lower (0.87). Considerably lower accuracy of NIRS prediction was recorded for crude fat where the  $R^2$  value was only 0.75 (Table 2). Constituents that are present in small quantities are known to fall under the category with the lowest accuracy and crude fat in hay is undoubtedly one of them. The poor predictive power of NIRS for fat can be ascribed also to the heterogenous nature of crude fat in grassland forages (De

Boever *et al.*, 1996) which includes besides tryglicerides also waxes, pigments and other compounds (Kircgeßner, 1997). The reports on capacity of NIRS for prediction of crude fat are contradictory. Some studies (Berardo *et al.*, 1997, Lavrenčič *et al.*, 2001) reported significantly higher  $R^2$  than in present study (0.97 and 0.85) while in others the  $R^2$  was lower (0.56–0.57, De Boever *et al.*, 1996).

Determination coefficient of calibration equation for NEL was 0.89. The value is similar to literature data for other biological parameters of forages, like *in vivo* or *in vitro* digestibility (0.87 to 0.94; Norris *et al.*, 1976, Park *et al.*, 1997; Bruno-Soares *et al.*, 1998 and Lavrenčič *et al.*, 2001). Determination coefficients between *in vitro* assessed concentrations of NEL and concentrations, which were estimated on the basis of digestibilities from tables or published equations, were considerably lower (from 0.54 to 0.60, Babnik *et al.*, 2001).

Table 2. Accuracy of the NIRS calibration equations\*

Preglednica 2. Točnost umeritvenih enačb\*

	$R^2$	SEC	SECV
Dry matter Suha snov	0.87	4.6	6.1
Crude protein Surove beljakovine	0.98	3.6	4.7
Crude fibre Surova vlaknina	0.95	9.0	12.6
Crude fat Surove maščobe	0.75	3.1	3.6
Ash Pepel	0.94	4.7	6.4
NEL	0.89	0.15	0.17

\* Calibration equations were developed on the basis of representative samples which were selected on the basis of their spectral characteristics. Only the samples with the Mahalanobis distance smaller than 3.0 from the mean and bigger than 0.6 between neighbours were included. The number of selected samples was 110.

\* Umeritvene enačbe so bile razvite na podlagi reprezentativnih vzorcev, ki so bili izbrani na podlagi značilnosti njihovih spektrov. Vključeni so bili le vzorci z Mahalanobisovo razdaljo manjšo od 3,0 od povprečja in večjo od 0,6 med sosednjimi vzorci. Število izbranih vzorcev je bilo 110.

ABREVIATIONS:  $R^2$  – coefficient of determination; SEC – standard error of calibration; SECV – standard error of cross validation

OKRAJŠAVE:  $R^2$  – koeficient determinacije; SEC – standardna napaka umeritve; SECV – standardna napaka navzkrižnega preverjanja

Reliability of NIRS method was tested on samples which were on the basis of Mahalanobis distance excluded from the calibration set (Table 3). Data are presented as deviations of NIRS predictions from the reference values which were obtained using wet chemistry or *in vitro* method. With exception of crude fat, more than 50% of predictions lied within repeatability limits (RL %) of reference methods. We should have in our mind, that repeatability limits are referred to results obtained using the same reference method in the same laboratory by the same operator using the same equipment. They are usually used to test the duplicates and therefore the criteria are relatively strong. Acceptable limits (AL), which are in case of chemical constituents defined by reproducibility limits, are much higher. They are referred to differences obtained using the same reference method in different laboratories by different operators using different

equipment. The criteria is met if 95% of results lies within these limits (AL %) and it is obvious, that NIRS method meets the criteria for dry matter, crude protein and crude fat, while the value for crude fibre is close to the target value (Table 3). Having in mind that reproducibility limits are referred to the results obtained by the same method and that two different methods (wet chemistry and NIRS) were compared in present experiment, also the result for crude fibre can be considered as satisfactory. The criteria of acceptable limits for ash content were not fulfilled and this shows that the use of NIRS for ash determination is limited (De Boever in sod., 1996; Lavrenčič in sod., 2001) and more attention is needed.

Table 3. Indicators of reliability of NIRS method for prediction of chemical composition (in  $\text{g kg}^{-1}$  DM) and energy value (in  $\text{MJ kg}^{-1}$  DM) of hay<sup>a)</sup>

Preglednica 3. Kazalci zanesljivosti metode NIRS za napovedovanje kemične sestave (v  $\text{g kg}^{-1}$  SS) in energijske vrednosti (v  $\text{MJ kg}^{-1}$  SS) mrve<sup>a)</sup>

	AID	MID	RL	RL %	AL	AL %
Dry matter Suha snov	6.5	22.5	7.6	60.4	29.0	100
Crude protein Surove beljakovine	3.7	-21.7	3.9	64.6	16.8	97.9
Crude fibre Surova vlaknina	8.5	30.2	9.3	58.3	19.5	93.7
Crude fat Surove maščobe	3.1	-9.6	2.0	45.8	7.9	95.8
Ash Pepel	5.1	21.4	5.0	56.2	10.1	87.5
NEL	0.12	0.39	/	/	0.30	95.8

<sup>a)</sup>Indicators of reliability are based on samples which were on the basis of Mahalanobis distance excluded from calibration procedure (n= 48)

<sup>a)</sup>Kazalci zanesljivosti temeljijo na vzorcih, ki so bili na podlagi Mahalanobisove razdalje izločeni iz postopka kalibracije (n= 48)

ABBREVIATIONS: AID – average individual deviation of predicted value from reference value ( $\text{AID} = \sum |d_i| / n$ ), where  $d_i$  is individual deviation of predicted value from reference value; MID – maximal individual deviation of predicted value from reference value; RL – Repeatability limits of reference methods. The absolute difference between two independent results obtained using the same reference method on identical test material in the same laboratory by the same operator using the same equipment within a short time should in not more than 5% of cases exceed RL. For CP RL is  $2.28 \text{ g kg}^{-1} + 0.0147 \text{ CP}$  (ISO 5983:1997(E)), for CF is 3% of individual reference value (73/46/EEC), for crude fat  $2 \text{ g kg}^{-1}$  (for samples containing less than  $50 \text{ g of crude fat kg}^{-1}$ ; 98/64/EC) and for ash  $5 \text{ g kg}^{-1}$  (for samples containing from 50 to 200 g of crude ash  $\text{kg}^{-1}$ ; ISO 5984–1978 (E)); RL % – percentage of samples within repeatability limits of reference method; AL – Acceptable limits. AL were defined as reproducibility limits. The absolute difference between two independent results obtained using the same reference method on identical test material in different laboratories by different operators using different equipment should in not more than 5% of cases exceed reproducibility limit. Reproducibility limit for CP is  $12.8 \text{ g kg}^{-1} + 0.0361 \text{ CP}$  (ISO 5983:1997(E)). As reproducibility limits for other constituents of forages are not available they were estimated on the basis of ratio between reproducibility and repeatability limits for available feedstuffs (ISO 6865: 2000(E), ISO 6492: 1999(E), ISO 5984: 2002(E)) and repeatabilities defined in EU directives for crude fibre and fat (73/46/EEC, 98/64/EC) or standard for ash (ISO 5984–1978 (E)). AL for NEL concentration is our own estimate; AL % – percentage of samples within acceptable limits

OKRAJŠAVE: AID – povprečno individualno odstopanje napovedane vrednosti od referenčne vrednosti ( $\text{AID} = \sum |d_i| / n$ ), kjer je  $d_i$  individualno odstopanje napovedane vrednosti od referenčne vrednosti; MID – največje individualno odstopanje napovedane vrednosti od referenčne vrednosti; RL – meje ponovljivosti referenčnih metod.

Absolutna razlika dveh neodvisnih rezultatov dobljenih z isto referenčno metodo na enakem preizkusnem materialu v istem laboratoriju z istim osebjem in opremo v kratkem času ne bi smela več kot v 5 % preseči RL. Za SB so RL  $2,28 \text{ g kg}^{-1} + 0,0147 \text{ SB}$  (ISO 5983:1997(E)), za SVI so 3 % od posamezne referenčne vrednosti (73/46/EEC), za surove maščobe  $2 \text{ g kg}^{-1}$  (za vzorce ki vsebujejo manj kot 50 g of maščob  $\text{kg}^{-1}$ ; 98/64/EC) in za pepel  $5 \text{ g kg}^{-1}$  (za vzorce, ki vsebujejo od 50 do 200 g pepela  $\text{kg}^{-1}$ , ISO 5984–1978 (E)); RL % – odstotek vzorcev znotraj mej ponovljivosti referenčne metode; AL – sprejemljive meje. AL so bile definirane kot meje obnovljivosti. Absolutna razlika dveh neodvisnih rezultatov dobljenih z isto referenčno metodo na enakem preizkusnem materialu v različnih laboratorijsih z različnim osebjem in opremo ne bi smela več kot v 5 % preseči mej obnovljivosti. Meje obnovljivosti za SB so  $12,8 \text{ g kg}^{-1} + 0,0361 \text{ SB}$  (ISO 5983:1997(E)). Ker meje obnovljivosti za druge sestavine voluminozne krme niso dostopne smo jih ocenili na podlagi razmerja med dostopnimi mejami obnovljivosti in ponovljivosti za druga krmila (ISO 6865: 2000(E), ISO 6492: 1999(E), ISO 5984: 2002(E)) in ponovljivosti, ki jih določajo smernice EU za surovo vlaknino in surove maščobe (73/46/EEC, 98/64/EC) oz. standard za pepel (ISO 5984–1978 (E)). AL za vsebnost NEL je naša ocena; AL % – odstotek vzorcev znotraj sprejemljivih meja

Predicted NEL values deviate from *in vitro* determined values on average for  $0.12 \text{ MJ kg}^{-1}$  DM (Table 3). The maximal deviation was  $0.39 \text{ MJ kg}^{-1}$  DM. Both values were considerably lower than corresponding values for predictions based on digestibility coefficients from DLG tables (0.29 and  $1.12 \text{ MJ NEL kg}^{-1}$  DM) or GFE equations (0.27 and  $1.59 \text{ MJ NEL kg}^{-1}$  DM) (Babnik *et al.*, 2001). More than 95% of samples lied within acceptable limits (AL) of  $\pm 0.3 \text{ MJ NEL kg}^{-1}$  DM (Table 3). The results suggest a comparative advantage of the NIRS method over the estimates based on chemical composition and equations from literature or digestibility factors from tables.

#### Distribution of samples which were included in calibration equation and accuracy of prediction with regard to NEL concentration

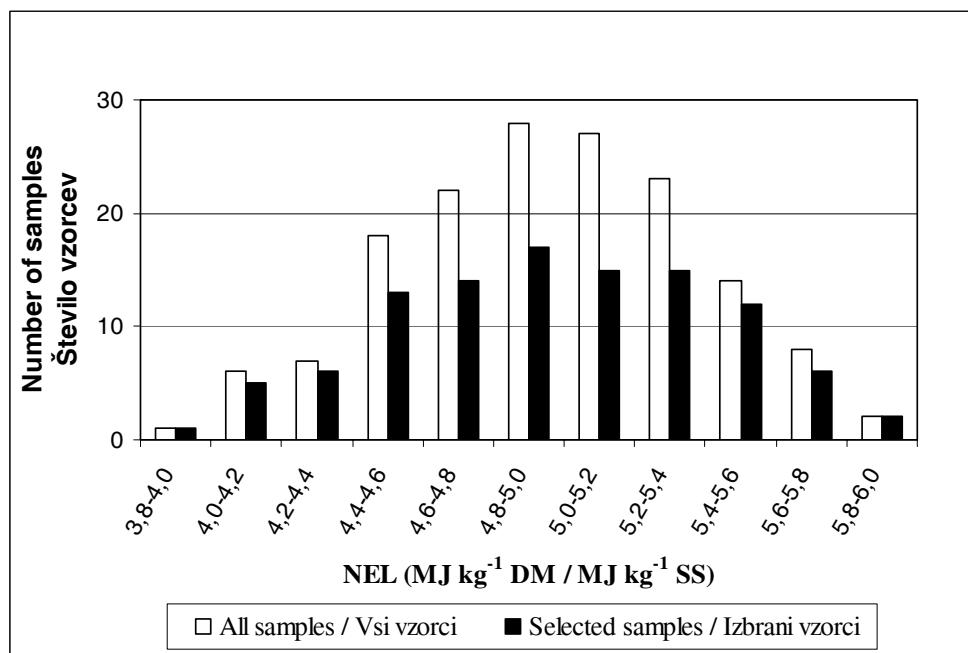


Figure 1. Distribution of total number of samples and number of samples which were selected for NIRS calibration with respect to NEL concentration.

Slika 1. Porazdelitev vseh vzorcev in vzorcev, ki so bili izbrani za umerjanje NIRS glede na vsebnost NEL.

Based on Mahalanobis distance only 110 from total 158 hay samples were selected for development of calibration equations. The number of selected samples is close to findings of Windham *et al.* (1989) that 50 to 100 samples are needed for narrow-based NIRS calibrations. However, although the number of selected samples seems adequate, it is obvious that the sample distribution is not optimal (Figure 1). While the number of samples in the range from 4.4 to 5.6 MJ NEL kg<sup>-1</sup> DM was large, only few samples lied in ranges below 4.4 MJ and above 5.6 MJ NEL kg<sup>-1</sup> DM. It seems that suboptimal distribution pattern does not affect the reliability of NEL predictions within individual classes considerably (Figure 2). Compared to classes with large number of samples, only slightly higher average individual deviation of predicted values from reference values was observed in class from 5.6 to 5.8 MJ NEL kg<sup>-1</sup> DM. On the other hand, in class from 4.0 to 4.2 MJ NEL kg<sup>-1</sup> DM, which was also characterised by small number of samples, the prediction of NEL was even better than on average. Some care is needed in interpretation of these data. We have to be aware that both extreme classes were tested only on the basis of few samples.

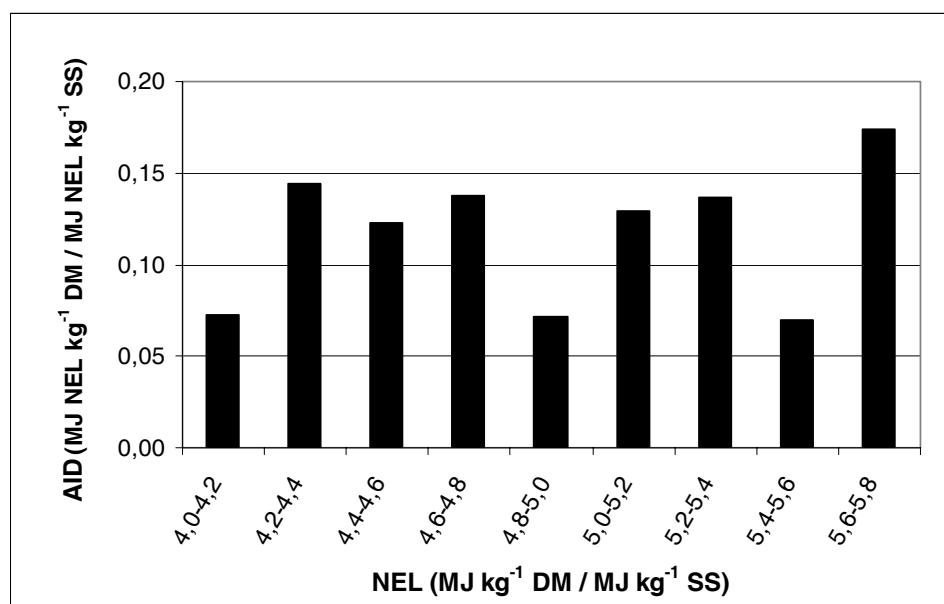


Figure 2. Average individual deviations of predicted NEL concentrations from reference values with respect to NEL concentration.

Slika 2. Povprečna individualna odstopanja napovedanih vsebnosti NEL od referenčnih vrednosti glede na na vsebnost NEL.

## CONCLUSIONS

It is concluded that NIRS is rapid and reliable technique for the determination of chemical composition of hay samples. With exception of ash deviations from reference methods are comparable to those which are expected by the use of the same reference methods in different laboratories. NIRS has a high ability to predict *in vitro* assessed NEL concentration ( $R^2 = 0.89$ ). More than 95% of samples lied within acceptable limits of  $\pm 0.3$  MJ NEL kg<sup>-1</sup> DM. With respect to prediction of nutritive value NIRS has an important comparative advantage over the methods based on chemical composition and digestibility factors from tables.

## POVZETEK

Namen raziskave je bil preučiti zanesljivost bližnje infrardeče refleksijske spektroskopije (NIRS) pri napovedovanju kemične sestave in vsebnosti NEL. Stoseminpetdesetim vzorcem mrve z znano kemično sestavo in *in vitro* ocenjeno vsebnostjo neto energije za laktacijo (NEL) smo izmerili spekture reflektirane bljižnje infrardeče svetlobe. Reflektirano bližnjo infrardečo svetlubo vzorcev smo merili v 8 nm intervalih v razponu valovnih dolžin od 1100 do 2500 nm. S pomočjo analize glavnih komponent (PCA) smo število spektralnih spremenljivk zmanjšali na neodvisne vire variabilnosti in jih pretvorili v številsko obliko. S pomočjo regresijske metode modificiranih delnih najmanjših kvadratov (MPLS) smo razvili umeritvene enačbe za ocenjevanje kemične sestave in neto energije za laktacijo (NEL).

Vzorci mrve so se v sestavi in vsebnosti NEL med seboj zelo razlikovali (preglednica 1). Ocenjujemo, da smo z vzorci pokrili razpon, ki je značilen za mrvo s slovenskih kmetij. Največjo točnost umeritvenih enačb smo dosegli pri surovih beljakovinah ( $R^2 = 0,98$ ), surovi vlaknini ( $R^2 = 0,95$ ) in pepelu ( $R^2 = 0,94$ ), nekoliko slabšo pa pri suhi snovi ( $R^2 = 0,87$ ) in surovih maščobah ( $R^2 = 0,75$ ) (preglednica 2). Koeficient determinacije umeritvene enačbe za *in vitro* ocenjeno vsebnost NEL je bil razmeroma velik (0,89) in primerljiv z literurnimi podatki za lastnosti, ki so povezane z vsebnostjo NEL (*in vivo* ter *in vitro* prebavljenost).

Zanesljivost umeritvenih enačb smo testirali na vzorcih, ki so bili na podlagi Mahalanobisove razdalje izločeni iz postopka umerjanja NIRS. Za kazalnike zanesljivosti smo uporabili meje ponovljivosti (RL) in sprejemljive meje (AL) (pregl. 3; okrajšave). Približno 50 % z NIRS ocenjenih vrednosti je bilo znotraj meja ponovljivosti (preglednica 3). Če upoštevamo sprejemljive meje, je bilo pri suhi snovi, surovih beljakovinah, surovih maščobah in NEL znotraj sprejemljivih meja več kot 95 % vrednosti. Pri surovi vlaknini je bilo takih 93,7 % vzorcev, pri pepelu pa 87,5 %. Glede na to, da so sprejemljive meje določene za isto metodo, mi pa smo primerjali dve različni metodi, smo lahko z rezultati zadovoljni.

Ugotovili smo, da je povprečno individualno odstopanje z NIRS ocenjene vsebnosti NEL od *in vitro* ocenjene vrednosti le  $0,12 \text{ MJ kg}^{-1}$  SS, kar je skoraj trikrat manj, kot če vsebnost NEL ocenimo na podlagi kemične sestave in prebavljenostnih koeficientov iz tabel. Podobno velja tudi za največja odstopanja, ki v primeru NIRS metode niso presegla  $0,40 \text{ MJ kg}^{-1}$  SS.

Na podlagi Mahalanobisove razdalje je bilo za umerjanje NIRS od 158 vzorcev izbranih le 110. Ugotovili smo, da porazdelitev vzorcev glede vsebnosti NEL ni bila enakomerna. V razredih pod  $4,4 \text{ MJ kg}^{-1}$  SS in nad  $5,6 \text{ MJ kg}^{-1}$  SS se je nahajalo le malo vzorcev (slika 1). Analiza odstopanj z NIRS ocenjenih vrednosti od referenčnih vrednosti po razredih je pokazala, da majhno število vzorcev v teh razredih ni vplivalo na zanesljivost ocenjevanja vsebnosti NEL z NIRS metodo (slika 2).

Sklenili smo, da je NIRS hitra in zanesljiva metoda za določanje kemične sestave in energijske vrednosti mrve.

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## TOXIC AND GENOTOXIC POTENTIAL EVALUATION OF SOIL SAMPLES BY BIOASSAYS

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### ABSTRACT

Soil samples were obtained from Slovenian industrial region Šaleška dolina and aqueous leachates were prepared. The toxicity and genotoxicity potential of these aqueous fractions have been evaluated by *in vitro* toxicity and genotoxicity bioassays. Freshwater toxicity test (PROTOXKIT F<sup>TM</sup>) with a ciliate protozoan *Tetrahymena thermophila*, and genotoxicity comet assay using *Tetrahymena thermophila*, Caco-2 and HepG2 cell lines were performed. Biological data were completed through chemical analyses. It has been shown that physico-chemical analyses alone may not be sufficient to characterize soil hazards. To study soil ecotoxicity it is therefore necessary to take into consideration both, the physico-chemical analyses, toxicity and genotoxicity assays.

Key words: microbiology / toxicity / genotoxicity / soil samples / *in vitro* bioassays / Slovenia

### VREDNOTENJE STRUPENOSTI IN GENOTOKSIČNOSTI VZORCEV ZEMLJE Z BIOTESTI

### IZVLEČEK

Na industrijskem področju Slovenije, v Šaleški dolini, smo vzorčili zemljo in pripravili vodne izlužke zemlje iz štirih vzorčnih mest. Toksični in genotoksični potencial vodnih izlužkov smo ovrednotili z uporabo *in vitro* biotestov za toksičnost in genotoksičnost. Izvedli smo test toksičnosti s sladjkovodno praživaljo, mitogenerjem, *Tetrahymena thermophila*, s t.i. PROTOXKIT F<sup>TM</sup> testom in test genotoksičnosti: kometni test s praživaljo *Tetrahymena thermophila*, in Caco-2 ter HepG2 celičnima linijama. Rezultate biotestov smo dopolnili s fizikalno-kemijskimi analizami. Za relevantnejše ekotoksikološko vrednotenje vzorcev zemlje je pomembno združiti fizikalno-kemijske analize in teste toksičnosti in genotoksičnosti.

Ključne besede: mikrobiologija / toksičnost / genotoksičnost / vzorci zemlje / *in vitro* biotesti / Slovenija

### INTRODUCTION

Soils are increasingly becoming sinks for a wide range of hazardous chemicals generated by human activities. These include aromatic compounds coming from black coal and lignite-mining, pesticides from agricultural soil contamination and heavy metals, solvents, and other industry related chemicals. Some are potent carcinogens and because of their solubility tend to accumulate in soils where their decomposition is mainly due to microbial action (Majer *et al.*,

2002). These hazardous compounds and their degradation metabolites endanger human health, soil and aquatic ecosystems by directly affecting soil biota or after runoff or leaking through soil, they endanger water or groundwater biota. Hazard and risk assessment of polluted soil samples is usually performed by means of physical and chemical measurements, but chemical analyses alone may not be enough due to the fact that:

- 1) are restricted to a limited list of defined compounds,
- 2) some measurements are based on sum parameters to determine broader classes of chemical compounds (e.g. total hydrocarbons or PAHs) with a high heterogeneity of toxic properties for environmental targets, and
- 3) bioavailability of organic and inorganic pollutants can vary (Bispo *et al.*, 1999).

Risk assessment based on ecotoxicity data for the soil compartment is rare and insufficient and as such the risk assessment is highly uncertain. The assessment of synergistic or antagonistic effects of the potentially (geno)toxic substances present in the soil, based on the assessment of the substance concentrations determined by physico-chemical analyse is very difficult (Debus and Hund, 1997). Thus alternative methods are needed such as biological tests (bioassays), which consist of exposing of living organisms to polluted materials. Two ways are already available to assess soil ecotoxicity (Bispo *et al.*, 1999). First, soil biota can be used: bacteria (Majer *et al.*, 2002), earthworms, collembolans (Zang *et al.*, 2000; Žnidaršič *et al.*, 2003, Drobne *et al.*, 2005), and plants (Ma, 1983). The use of these organisms has been standardised to assess the effects of pure substances on soil biota (ISO 11269-1 and -2, 1993; ISO 11268-1 and -2, 1994; ISO 11240-1 and -2, 1997), the draw back being that organisms can adapt to polluted soil materials. Bioassays, which use entire solid matrix, are both time and space consuming and therefore expensive. The second way to assess the soil ecotoxicity is to perform bioassays on soil water leachates (Beakert *et al.*, 1999, Bekaert *et al.*, 2002), where a response can be obtained rather quickly.

In this study the (geno)toxicity of soil samples was determined by bioassays on soil water leachates. The aim of these preliminary studies was to introduce indirect exposure procedures to assess soil ecotoxicity in combination with physico-chemical analyses.

## MATERIAL AND METHODS

### **Soil samples**

Coal and lignite-mining and coal firing, industry, nearby thermal power plant (TPP) and lead smelter (Žerjav), the traffic, and farming, left ecotoxicological consequence in Slovenian industrial-rural region called Šaleška dolina and nearby region, where four soil samples (Silova-sample 1, which is 8.2 km distant from TPP; Mali vrh-sample 2, 4.0 km distant from TPP; Lubela-sample 3, 5.0 km distant from TPP and Andraž-sample 4, which is 6.4 km distant from TPP) were collected according to ISO standard procedure (ISO 11464, 1994; ISO 10381, 1996) at one occasion in december 2003 (9.12.2003). Soil sampling and physico-chemical analysis were performed in the laboratories of ERICo Velenje. Soil samples were dried and sieved according to standard procedure (ISO 11464) and afterwards stored at -20 °C for further analyses. Since the thermal power plant is the main source of heavy metal pollution in the valley, where Pb, Cd, Zn, Hg beside nitrogen oxides and sulphur oxides, are released into the environment as dust particles, heavy metal contents were determined and physico-chemical analyses were performed according to recommended ISO standards (SIST ISO: 5664, 5666, 6468, 10523, 11083, 1996; SIST EN 27888, 1998; SIST EN ISO 10304-2, 1998; SIST ISO: 8245, 9562, 2000; SIST DIN 38406-29, 2000). For biotesting water soil leachates were prepared (4.2.2004). Leaching was performed for 24h in a linear shaking apparatus at 25±1 °C and the

suspension was allowed to settle for 24h at 4 °C. The supernatant was then used for biotesting the next day (Békaert *et al.*, 1999).

### **In vitro comet assay with *Tetrahymena thermophila***

*T. thermophila* (amicronucleate strain) was originally obtained from Microbiotest (Belgium) as part of the Protox F™ kit. *T. thermophila* cells were cultivated in a semidefined medium for protozoa (Schultz, 1997) at 30 °C for 72h. The cells grow in liquid cultures as motile unicellular ciliates below the surface of the medium. Before incorporation of the cells into the agarose layers, the cells were concentrated by centrifugation in Falcon tubes for 3 min at 300 x g and 4 °C.

The dye-exclusion test with Trypan blue (Wilson, 1986) was used to examine the viability of cells before the comet assay was performed.

The modified version of the original Singh *et al.* (1988) protocol, the alkaline comet assay on protozoa was preformed according to the method described by Lah *et al.* (2004). In order to assess genotoxic potential of potentially bioavailable fraction of soil, the cells of *T. thermophila* were exposed to water leachates of soil. The cells were also exposed to 0.9% solution of NaCl (a negative control) and to 500 µM hydrogen peroxide, H<sub>2</sub>O<sub>2</sub> (a positive control).

### **In vitro comet assay with Caco-2 and HepG2 cell line**

Epithelial colon cancer cells (Caco-2, obtained from Instituto Zooprofilatico Esperimental, Brescia, Italy) and human hepatoma cell line (HepG2 cells, obtained from prof. dr. Knasmüller, Institute of Cancer Research of The Univeristy of Vienna) were grown in multilayer culture at 37 °C in hummified atmosphere of 5% CO<sub>2</sub> in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal calf serum (FCS) and antibiotic (0.1% gentamycin) in culture flasks for 8 days (cell density: 10<sup>6</sup>–10<sup>8</sup> cells/mL). Medium was changed every 2 days. Single cell suspensions were prepared with 0.25% trypsin-EDTA solution, passed through injection needle for several times to achieve single cell suspension and finally resuspended in DMEM medium, supplemented with 10% FCS medium (Lah *et al.*, 2005).

The dye-exclusion test with Trypan blue (Duthie *et al.*, 1997a) was used to examine the viability of cells before the comet assay was performed.

Briefly, to achieve a uniform background rough microscope slides were first coated with up to 400 µL of 1% normal melting point agarose (NMP) and left to air dry overnight. The supportive (second) agarose layer (0.6% NMP agarose) was solidified on ice and the collected Caco-2 or HepG2 or protozoa cells were immobilized in the third layer at concentrtrion of approximately 1–2 ×10<sup>5</sup> cells/ml. After removing the cover glasses, the slides were covered with 500 µL of 0.5% LMP agarose (the fourth layer).

Four layered slides with incorporated cells were first submerged into water samples, negative control and positive control solutions for 20 min and followed by 1h incubation in alkaline lysis buffer. The slides were submerged in electrophoretic buffer (pH > 13) to unwind the nuclear DNA for 1h and then subjected to electrophoresis in the same buffer. The electrophoresis was carried out at 2V/cm and 300 mA; time of electrophoresis depended on cell type (30minutes for Caco-2 and HepG2 cells, and 5 minutes for *Tetrahymena thermopila* cells). Following electrophoresis the gels were neutralized in 400 mM Tris-HCl pH 7.5 for 15 min. The damaged DNA travelled toward the anode during electrophoresis and formed an image of a “comet” tail. After staining the slides with ethidium bromide (20 µg/mL) the comets were detected and quantified as described below.

## Data collection and statistical analyses of the results of the comet assay

Quantitative analyses of nuclear DNA damage in *Tetrahymena thermophila*, Caco-2 and HepG2 cells was done by 20 x objective magnification with an epifluorescence microscope (Olympus BX 50) using a BP 515–560 nm filter and BA 590 nm barrier filter and digital camera (Hamamatsu Orca 2) connected to a computer, and the comets were scored using Komet 5.0 Computer Software (Kinetic Imaging, 2001).

Olive tail moment (OTM) was chosen as the most relevant measure of genotoxicity. Tail length and the percentage of DNA in comet tails and heads were collected. These values were used to calculate OTM, using the relationship: OTM = (tail mean – head mean) X tail % DNA / 100 in arbitrary units (Olive *et al.*, 1990).

Images of 50 comets were collected from each of two replicate slides per sample, OTMs were calculated using SAS/STAT statistical software (version 8e; SAS Institute, Cary, NC, 2000) and software package Graph Pad Prism 3.0 (version 3.02, GraphPad Software Inc., 2000). Descriptive statistics were determined by the MEANS procedure. OTM records were tested for normal distribution with the UNIVARIATE procedure. Bauer *et al.* (1998) suggested that the distribution of OTMs obeys a Chi-square ( $\chi^2$ ) distribution. The chi-square distribution, which is a special case of the gamma distribution, fitted well to our data. As a consequence, data were analysed by the GENMOD procedure (Generalised Linear Models) which allows distributions other than a Gaussian one. Statistically significant differences between groups were evaluated by the linear contrast method.

## Fresh water toxicity test with ciliated protozoan *Tetrahymena thermophila* (PROTOXKIT F<sup>TM</sup>)

A 24h growth inhibition test was performed with the ciliate *Tetrahymena thermophila*. The test is based on the turnover of substrate into ciliate biomass. While normal proliferating cell cultures clear the substrate suspension in 24h, inhibited culture growth is reflected by remaining turbidity. Optical density measurements of the turbidity quantify the degree of inhibition. To minimize toxic buffering of e.g. heavy metals and/or reactive and highly lipophilic substances (by binding the organic components in the medium which may interfere with the bioavailability of the toxicants) the PROTOXKIT medium had an organic content more than a factor 100 lower than that of the proteose-peptone media, used in conventional *Tetrahymena* tests (Microbiotest, Protox F<sup>TM</sup>, Belgium).

## Data treatment – estimation of the 24h EC50 for PROTOXKIT F<sup>TM</sup> test

According to the kit instructions (Microbiotest, Protox F<sup>TM</sup>, Belgium), the mean for the two parallels for each toxicant/sample dilution and the control was calculated. Then the difference between the mean OD at T0 and T24 for each toxicant/sample dilution ( $\Delta OD_{C1-Cx}$ ) and for the control ( $\Delta OD_{C0}$ ) was calculated. The % of inhibition for each toxicant/sample dilution was estimated by the following equation:

$$\% \text{ inhibition}_{(C1-Cx)} = \left( 1 - \frac{(\Delta OD_{C1-Cx})}{(\Delta OD_{C0})} \right) \times 100$$

## RESULTS AND DISCUSSION

In this study, *T. thermophila* was taken as a test organism because it represents an ideal model for both toxicological and ecotoxicological studies (Sauvant *et al.*, 1999) and it can be easily grown in pure cultures. Protozoa have also proved as sensitive organisms to environmental

changes (Nicolau *et al.*, 1999, Chen and Leick, 2004). *Tetrahymena thermophila* has been used as a microbial model due to its typical eukaryotic characteristics. Its ultrastructure, cell physiology, development, biochemistry, genetics and molecular biology have been extensively studied (Asai and Forney, 2001) and the organisms are extensively used for toxicity testing because of their sensitivity to heavy metals (Chen and Leick, 2004).

Short-term tests with cultivated mammalian cells are widely used for detection of potential environmental mutagens and carcinogens. Since numerous compounds exert their genotoxic and carcinogenic effects only after metabolic activation, it is important that indicator cell lines possess critical enzymes. For this reason, indicator cells competent in xenobiotic metabolism would be desirable (Glatt *et al.*, 1990). HepG2 cells are a highly differentiated human hepatoma cell line, which has retained many of the specialized functions usually lost upon culturing and as such represent a suitable *in vitro* system for studying drug metabolism and (geno)toxicity in man (Duverger-van Bogaert *et al.*, 1993). The other chosen indicator cell line was Caco-2. Even though this cell line appears not to posses all the desirable xenobiotic metabolising enzymes, it exhibits different degrees of specialisation and enterocyte like functions. The intestinal epithelium permeability, which resembles the Caco-2 cell line, is a critical characteristic that determines the rate and extent of human absorption and ultimately the bioavailability of a xenobiotic compound (Duthie *et al.*, 1997b).

Toxic substances that could be found in soil and water are numerous. Using chemical and physical analyses, we tried to estimate the concentrations of some of the most (geno)toxic substances like heavy metals, pesticides, volatile chlorinated aliphatic carbohydrates and aromatic substances. Some of the general parameters like (pH, specific electric conductivity, etc.) were also estimated, because their contribution to overall (geno)toxicity is great. Sulphate, phosphate, nitrogen and carbon contents are also very important parameters in the field of ecotoxicological evaluation of environmental samples. Studies on toxic effects of trace metals on both soil (micro)organisms and native plants (Glasenčnik *et al.*, 2004; Kugonič *et al.*, 2004) in field conditions are limited and differ from the studies in the laboratory conditions. Studies of the toxic effects of metals should take into consideration: the characteristics of the toxic metal (metal chemical forms) bioavailability, mechanisms of toxic metal action, interactions with other metals, cumulative effects, etc. Some of the main problems in such studies are that soil properties influence the rates of metal transfers to plants and other organisms and no chemical or toxicant interactions are taken into account, etc. (Patra *et al.*, 2004).

The total metal concentration of heavy metals in soil samples is not a reliable indicator of metal concentration extracted with water, therefore it is expected that the results of (geno)toxicity assays of complex mixtures like soil are strongly influenced by the method of sample preparation and extraction, because chemical and physical properties of constituents, including genotoxic compounds, in the mixture may differ substantilly. In most of the studies assessing (geno)toxicity, leachates of soil samples were prepared prior to the assay. The leachates from soil samples were generally made by shaking soil samples with aqueous solvents and preferably not filtered (Bekaert *et al.*, 2002), so we followed this step in our study too.

Sample 2 (followed by sample 3, 4 and 1) showed the highest heavy metal contents according to chemical and physical analyses of water soil leachates, presented in Table 1. The concentrations of all parameters for soil leachates measured in table 1 were bellow the detection limit of chosen chemical analytical methods for all sampling locations according to recommended standard method SIST DIN 38406-29 (2000) and ATSDR/CERCLA (2003). However the measurements of As and Cd contents in all four soil samples, exceeded the critical value for As ( $72.2 \text{ mg kg}^{-1}$ ) and the warning immision value for Cd ( $3.6 \text{ mg kg}^{-1}$ ) only in sample 2. Limit immision value for Ni ( $62.3 \text{ mg kg}^{-1}$ ) was exceeded in sample 1 (Table 3, Uredba., Ur. L. RS, 1996; Stropnik, 2003).

Table 1. Heavy metal contents and other physico-chemical parameters of water soil leachates  
 Preglednica 1. Prikaz rezultatov vsebnosti težkih kovin in fizikalno-kemijskih analiz vodnih izlužkov zemlje

PARAMETER	units	sample point			
		Sample 1	Sample 2	Sample 3	Sample 4
Arsenic – As	µg/L	1.23	1.82	1.39	1.40
Cobalt – Co	µg/L	0.98	2.83	1.91	0.26
Chromium – Cr	µg/L	0.89	1.29	0.87	0.75
Molybdenum – Mo	µg/L	1.06	15.00	2.45	1.38
Selenium – Se	µg/L	2.01	2.17	2.01	1.54
Silver – Ag	µg/L	0.54	0.55	0.59	0.53
Copper – Cu	µg/L	5.17	9.67	4.87	13.20
Zinc – Zn	µg/L	4.32	6.69	6.50	10.60
Cadmium – Cd	µg/L	0.66	0.73	0.70	0.65
Nickel – Ni	µg/L	5.74	2.72	1.75	4.02
Lead – Pb	µg/L	3.25	3.75	3.73	3.61
Specific Electric Conductivity – SEC (T= 25.0 °C)	µs/cm	391.0	240.0	235.0	86.0
T (SEC)	st.C	20.0	20.6	20.0	19.6
Volatile chlorinated CH – VCCH	µg/L	<0.5	<0.5	<0.5	<0.5
1,1,1-trichloroethane	µg/l	<0.5	<0.5	<0.5	<0.5
1,1,2,2-tetrachloroethylene	µg/l	<0.5	<0.5	<0.5	<0.5
1,1,2-trichloroethylene	µg/l	<0.5	<0.5	<0.5	<0.5
Dichloromethane	µg/l	<0.5	<0.5	<0.5	<0.5
Tetrachloromethane	µg/l	<0.5	<0.5	<0.5	<0.5
Trichloromethane	µg/l	<0.5	<0.5	<0.5	<0.5
Chloride	mg/L	2.92	1.56	1.40	1.25
Nitrate – nitrogen	mg-N/L	<0.2	<0.2	<0.2	<0.2
Nitrite – nitrogen	mg-N/L	<0.3	<0.3	<0.3	<0.3
Sulphate	mg/L	2.98	3.44	1.81	1.31
pH	/	7.76	8.21	8.20	7.71
T (pH)	°C	19.0	19.7	19.2	19.9
Chromium – Cr (VI)	mg/L	<0.01	0.02	0.01	<0.01
Volatile aromatic CH – BTX	µg/L	<10	<10	<10	<10
Benzene	µg/L	<10	<10	<10	<10
Ethilbenzen	µg/L	<10	<10	<10	<10
Xylene	µg/L	<10	<10	<10	<10
Toluene	µg/L	<10	<10	<10	<10
Trimethylbenzene	µg/L	<10	<10	<10	<10
Ammonia nitrogen	mg N/L	1.7	2.1	2.3	1.1
Mercury – Hg	µg/L	<0.5	<0.5	<0.5	<0.5
total organic carbon – TOC	mg C/L	47.8	40.5	23.1	20.5
Adsorptive Organic Halogens – AOX	µg Cl /L	11.00	11.00	<10	<10
Total cyanide	mg/L	<0.05	<0.05	<0.05	<0.05

A set of bioassays was performed on the leachates obtained from four soil samples to assess the toxicity and genotoxicity. Because of the problems of extractability of heavy metals and other possible genotoxic compounds mentioned above, different results from different bioassays using different cell types were expected. The results of the toxicity assay PROTOXKIT F<sup>TM</sup> test with water leachates presented in Table 2, showed the estimated EC50 value of 69.99% for sample 4. For all the other samples the EC50 values were not detected due to low toxicity.

Table 2. Optical density measurements (OD at 440 nm) and estimation of EC50 value with PROTOXKIT F<sup>TM</sup>Preglednica 2. Prikaz meritev optične gostote (OD pri 440 nm) in EC50 s testom PROTOXKIT F<sup>TM</sup>

Sample point	Sample concentration %	OD(»a«)			OD (»b«)			% inhibition	EC50
		T0	T24	T48	T0	T24	T48		
Sample 1	control	0.613	0.567	0.167	0.683	0.571	0.159	/	
	100	0.634	0.634	0.223	0.655	0.595	0.208	11.55	
	50	0.690	0.604	0.203	0.678	0.567	0.201	0.62	
	25	0.725	0.614	0.184	0.607	0.566	0.188	1.03	ND
	12.5	0.656	0.610	0.196	0.646	0.576	0.188	5.36	
	6.25	0.688	0.585	0.420	0.688	0.600	0.164	18.35	
Sample 2	control	0.685	0.579	0.154	0.682	0.588	0.185	/	
	100	0.662	0.496	0.159	0.685	0.506	0.176	4.18	
	50	0.742	0.555	0.150	0.616	0.579	0.187	0.68	
	25	0.675	0.547	0.165	0.683	0.551	0.145	-1.95	ND
	12.5	0.646	0.534	0.206	0.673	0.550	0.159	7.20	
	6.25	0.678	0.535	0.227	0.714	0.577	0.174	3.60	
Sample 3	control	0.641	0.549	0.167	0.648	0.518	0.125	/	
	100	0.711	0.505	0.157	0.643	0.519	0.149	-5.12	
	50	0.685	0.513	0.159	0.662	0.533	0.137	-5.42	
	25	0.689	0.534	0.150	0.690	0.564	0.179	-5.32	ND
	12.5	0.656	0.582	0.165	0.705	0.525	0.227	3.81	
	6.25	0.639	0.556	0.169	0.666	0.528	0.257	11.84	
Sample 4	control	0.666	0.557	0.167	0.675	0.578	0.159	/	
	100	0.818	0.727	0.697	0.797	0.750	0.578	65.91	
	50	0.686	0.611	0.318	0.762	0.677	0.403	34.58	
	25	0.677	0.596	0.331	0.649	0.580	0.326	34.09	69.99%
	12.5	0.668	0.550	0.261	0.712	0.579	0.345	23.74	
	6.25	0.635	0.526	0.237	0.699	0.550	0.292	20.69	

a, b – parallels of dilutions of water leachates; ND- EC 50 not detected

The results of the comet assay on three different cell types are presented in Figure 1. The comet assay results with *Tetrahymena thermophila* cells showed the statistically significant increase of genotoxic potential according to negative control ( $p < 0.005$ ) for sample 1. The comet assay with Caco-2 and HepG2 cell lines showed increases of genotoxic potential according to negative control for samples 2 and 4, which however were not statistically significant. The negative results obtained by the comet assay on human cell lines and also in toxicity testing with PROTOX F<sup>TM</sup> test for samples 1, 2 and 3 could be explained by low extractability of heavy metals and other possible genotoxic compounds (like pesticides) by water.

Observations of potential metal genotoxicity in soil are rare and majority of metal (geno)toxicity are focused on soil-plant interactions trough metallic salts. Most metallic salts are effective poisons at particular concentrations, because they are able to bind to thiol groups and

induce spindle disturbances in the cells which can be detected by micronucleus assay on plant cells.

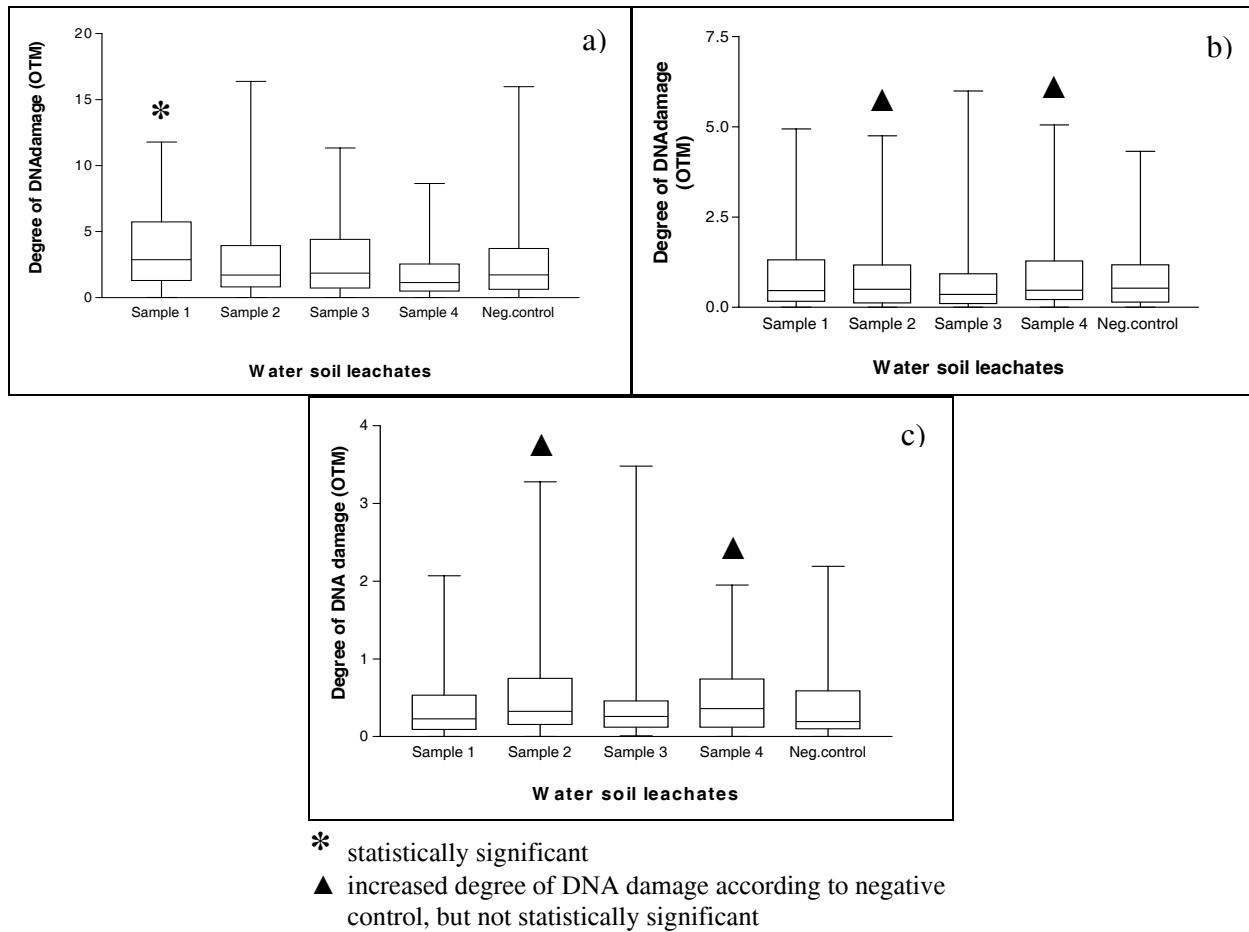


Figure 1. Nuclear DNA damage in *T. thermophila* (a), Caco-2 (b) and HepG2 (c) cells (represented as OTM) treated with water soil leachates. Results from 100 comets for each soil sample are shown as box-and-whiskers plots. The OTM values are shown as boxes that include 50% of the data. The top and bottom of the boxes mark 25<sup>th</sup> and 75<sup>th</sup> percentiles; the inner line marks the median value. 25% of the data above the 75<sup>th</sup> percentile and 25% of the data below the 25<sup>th</sup> percentile are marked as “whiskers” limited by the maximum or minimum values.

Slika 1. Poškodbe jadrne DNK na *T. thermophila* (a), Caco-2 (b) and HepG2 (c) celicah, izražene z RM-Olive (Repni Moment po Olivu) po izpostavitvi vodnim izlužkom zemlje. Rezultati 100 ocenjenih celic so prikazani z okvirji z ročaji. 50 % podatkov vrednosti RM-Olive so prikazane v okvirjih. Zgornji in spodnji ročaj predstavlja 25. in 75. percentil; notranja meja, pa mediano vrednost. 25 % podatkov se nahaja nad vrednostjo 75. percentila in 25 % pod vrednostjo 25. percentila, ki sta omejeni z maksimalno in minimalno vrednostjo RM-Olive.

Many researchers including Bekaert *et al.* (1999) agree that it is hard to establish the relationship between the contaminants and (geno)toxicity results because of interactions phenomena such as synergistic and antagonistic effects. While interpreting the data, the influence of natural substances extracted from the soil studied should not be excluded. A negative control soil with the same composition as samples studied would have been needed to distinctly state the genotoxic effects of present soil contaminates. As negative control with an identical composition to the soil sample is rarely found in environmental studies, the

identification of the kinds of pollutants responsible for (geno)toxicity is always difficult and can only be suggested. It can be frequently observed that genotoxic responses are registered in bioassays, while no (geno)toxic chemicals can be identified in the fraction studied. Many authors report on mutagenicity found in environmental samples, although no mutagenic compound could be detected following standard analytical methods (Maron in Ames, 1983; Donnelly *et al.* 1995), this underlines the fact that physical and chemical analyses are not always extensive and that other pollutants than the measured ones may be present in the tested sample and may be responsible for the (geno)toxicity observed.

Table 3. Heavy metal contents in soil samples for chosen locations accordnig to Slovenian regulations (Uredba., Ur. L. RS, 1996)

Preglednica 3. Vsebnosti težkih kovin v tleh na izbranih vzorčnih lokacijah in imisijske vrednosti težkih kovin v tleh povzeto po veljavnem predpisu (Uredba., Ur. L. RS, 1996)

	Cd mg kg <sup>-1</sup>	As mg kg <sup>-1</sup>	Ni mg kg <sup>-1</sup>
Limit immision value	1	20	50
Warning immision value	2	30	70
Critical Immision value	12	55	210
Sample 1	0.6	9.1	62.3
Sample 2	3.6	72.2	37.9
Sample 3	1.8	18.8	27.6
Sample 4	0.5	3.0	16.1

## CONCLUSIONS

A combined testing protocol for risk assessment using both biological and physico-chemical analyses provides more accurate information than the use of either method alone. Our future plan is to introduce a plant bioassay, *Tradescantia* micronucleus assay, for (geno)toxicity testing of soil samples and soil water extracts. This may give more relevant information on soil ecotoxicity. Since the extractability of heavy metals with water is expected to be very low, the tests on soil samples and water extracts need to be done in order to compare the results and to determine the most sensitive battery of bioassays suitable for soil ecotoxicity evaluations.

## POVZETEK

Onesnaženje zemlje vpliva tudi na vodni ekosistem in posledično na človeka. Vnos strupenih snovi (kot so: težke kovine, pesticidi, ipd.) v zemljo postaja vedno večji in mnoge od teh snovi imajo potencialni (geno)toksični in/ali kancerogni vpliv na živa bitja. Kemijske analize vzorcev zemlje in vodnih izlužkov zemlje nam ne povedo veliko o dejanskih bioloških učinkih prisotnih onesnaževal v okolju, zato je uporaba biotestov v spremeljanju stanja okolja zelo pomembna. Le biotesti nam lahko ponudijo odgovore o možnem sinergizmu, aditivizmu in drugih učinkih onesnaževal na živa bitja, iz česar lahko tudi izpeljujemo ocene tveganja človeka, ki se nahaja v takšnem okolju.

V Šaleški dolini, kjer je v preteklih letih prihajalo do onesnaženja zaradi premogovništva, bližine termoelektrarne in toplarne in danes prihaja do onesnaženja v glavnem zaradi prometa in kmetijstva, smo vzorčili zemljo. Pripravili smo vodne izlužke zemlje. Ker nam fizikalno-kemijske analize izlužkov pokažejo le prisotnost določenih snovi in njihove koncentracije, smo

žeeli v tej študiji preveriti še geno(tokičen) potencial izlužkov zemlje z biotesti. V ta namen smo izvedli test strupenosti s *Tetrahymeno thermophilo* (PROTOXKIT F<sup>TM</sup>) in kometni test (test genotoksičnosti) z istim organizmom in dvema celičnima linijama (Caco-2 in HepG2).

Fizikalno-kemijske analize niso pokazale preseženih mejnih koncentracij težkih kovin, pesticidov in njihovih presnovnih produktov ter drugih organskih spojin v vodnih izlužkih, glede na lestvico ATSDR/CERCLA (2003). Biotesti pa so pokazali strupenost enega vzorca (vzorec 4) in povečano stopnjo poškodb DNA glede na negativno kontrolo, ki je bila statistično značilna v primeru uporabe *Tetrahymene thermophila* kot testnega organizma, in statistično neznačilna za vzorca 2 in 4 v primeru uporabe celičnih kultur.

Menimo, da je potrebno za relevantnejše ekotoksikološko vrednotenje vzorcev zemlje in drugih vzorcev iz okolja, združiti fizikalno-kemijske analize okoljskih vzorcev s testi toksičnosti in genotoksičnosti.

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## **INCOME SITUATION OF AGRICULTURAL HOUSEHOLDS IN SLOVENIA AFTER EU ACCESSION: IMPACTS OF DIFFERENT DIRECT PAYMENTS POLICY OPTIONS**

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### **ABSTRACT**

Paper investigates income effects of different direct payments policy options after the accession of Slovenia to the EU by application of a static deterministic total income model for rural households in Slovenia (TIM). Model is based on actual income data of 120 agricultural households in Slovenia. With respect to the baseline situation before the accession and accession agreement, income situation of analyzed households is likely to improve under all analyzed policy scenarios. The estimated benefits are highest in case of the standard direct payments scheme, followed by basic flat-rate area payment option (entirely decoupled). Model results reveal also that 2003 policy reform will have redistributive impacts in favour of agricultural households engaged in extensive agricultural production.

Key words: agricultural households / EU enlargement / CAP reform / total income / income impacts / Slovenia

## **DOHODKOVNI POLOŽAJ KMEČKIH GOSPODARSTEV V SLOVENIJI PO PRISTOPU K EVROPSKI UNIJI: UČINKI RAZLIČNIH SHEM NEPOSREDNIH PLAČIL**

### **IZVLEČEK**

V prispevku je prikazana primerjava dohodkovnih učinkov različnih shem neposrednih plačil za vzorec 120 kmečkih gospodarstev v Sloveniji po pristopu k Evropski uniji. Rezultati so bili pridobljeni z uporabo statičnega determinističnega modela za oceno skupnega dohodka kmečkih gospodinjstev TIM. Na podlagi modelnih rezultatov ocenjujemo, da se bo dohodkovni položaj analiziranih kmečkih gospodarstev v primerjavi z izhodiščnim predpristopnim položajem najverjetneje izboljšal v primeru vseh analiziranih popristopnih scenarijev. Največje koristi so ocenjene v primeru implementacije standardne sheme neposrednih plačil. Druga dohodkovno najugodnejša pa je v celoti proizvodno nevezana čista regionalna shema. Na podlagi modelnih rezultatov je mogoče sklepati, da bo imela reforma neposrednih plačil iz leta 2003 ugodnejše učinke za proizvodno ekstenzivnejša kmečka gospodarstva.

Ključne besede: kmečka gospodarstva / širitev EU / CAP / skupna kmetijska politika / reforme / skupni dohodek / dohodkovni učinki / Slovenija

## INTRODUCTION

Accession process to the European Union (EU) has significantly changed the structure and scope of agricultural support in Slovenia (Rednak *et al.*, 2003). Direct payments (DP) became the most important element of agricultural policy with significant impacts on income of rural households. The accession process coincided with reform process of Common agricultural policy (CAP) of the EU. CAP reform, agreed in Luxembourg in June 2003 (EU News..., 2003), in terms of DP implies a gradual decoupling of support from production. In order to prevent short-term redistribution effects, member states can use various alternatives to retain part of this support production-linked. Thus, Slovenia had been faced with the decision which DP policy option to implement in i) immediate post-accession period (2004–2006) and ii) in period, when the 2003 CAP reform provisions should be enforced (implementation of CAP reform in 2007 at the latest).

In the immediate post-accession period either standard DP scheme (actual CAP accepted for old member states for the period from 2000 to 2006), or “simplified” DP scheme (“Simplified area payment scheme” or “SAPS”), a production decoupled area payment, was an option for Slovenia (Treaty of Accession, 2003). Besides Malta, Slovenia was the only new member state to opt for standard DP option in the immediate post-accession period (Council Regulation..., 2003). As CAP reform DP policy new member states are obliged to implement “regional flat-rate payment” (based on regional reference quantities), with an option to retain certain elements of the standard CAP scheme, which is production-coupled (EU News..., 2003; Council Regulation..., 2003). Slovenia will implement this scheme by 1<sup>st</sup> January 2007 at the latest.

This paper presents a comparison of economic impacts of different post-accession DP policy options on the level of agricultural households in Slovenia. Estimates were obtained by application of static deterministic total income model for rural households in Slovenia TIM (model TIM), developed by Erjavec *et al.* (2002), Oblak (2002) and Kožar *et al.* (2003). Taking into consideration the existing diversity of structure of total income of agricultural households in Slovenia (Erjavec *et al.*, 2002; Oblak, 2002), income effects are investigated also by different employment types (especially full-time farms) and income groups of households. Paper starts with a short description of analyzed data, model and policy scenarios. Model results are presented for the whole sample, by employment types and by total income groups. Discussion chapter gives final conclusions based on model results.

## METHODOLOGY: DATA, MODEL AND POLICY SCENARIOS

The model is based on the survey data from 120 agricultural households, complemented by selected secondary data (Statistical yearbook, 2002; Rednak, 2003). Households were proportionally sampled from four strata, i.e. employment types: full-time agricultural households (full-time farms), part-time agricultural households (part-time farms), self-employed agricultural households and non-agricultural rural households. Furthermore they were sampled from four municipalities, which lie in two regions different in terms of general economic standard and significance of agricultural sector in their economic structure: Pomurje (less developed) and Gorenjska (more developed) region. From each region two municipalities were chosen, one located in less favoured area for agricultural production. From each municipality 30 households were randomly sampled.\*

Static deterministic total income model TIM enables rough estimations of incomes by different sources and estimation of labour allocation on household level. It operates as a system

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\* Further details on data collection, sampling procedure and sample features are described in Erjavec *et al.* (2002) and Oblak (2002).

of four sub models for estimating yearly incomes by their source (income from agriculture, income from off-farm activities, income from self-employment activities and income from other sources) with additional sub model for estimating labour allocation. Basic model assumptions are following:

- Years 2001 and 2006 are considered as base year and as simulated post-accession year, respectively.
- Only policy changes in agricultural sector are considered. They are based on the accession agreements for Slovenia, which entail negotiated reference quantities, production quotas and negotiated funds committed for DP and rural development policies in year 2006 (Treaty of Accession, 2003; EU and enlargement, 2003).
- Prices of agricultural products in year 2006 are set according to the expert opinion on the expected decrease of overall price level (Kavčič and Erjavec, 2003) and are identical in all scenarios.
- Income impacts are considered in terms of real prices.
- Investigated households are assumed to have received the entire set and amount of CAP aids within their production limitations and natural conditions for agricultural production in base year 2001 and in year 2006.

Analyzed DP policy scenarios are in detail described in Table 1.

Table 1. Scenario description (EU and enlargement, 2003; Rednak, 2003; Statistical Yearbook, 2002; Erjavec *et al.*, 2004)

Preglednica 1. Opis scenarijev

Scenario – long name	Scenario – short name	Short description
Base year scenario	2001	Estimate of base year (2001) income situation of sample households.
Standard DP scheme	EUo	Standard CAP scheme as agreed for the period 2000–2006; different types of area and headage DP
Simplified area payment scheme	SAPS	Decoupled area payment – entire utilized agricultural area (UAA) eligible. Value estimated: <ul style="list-style-type: none"><li>• 237 € per hectare (ha) of UAA.</li></ul>
Basic flat-rate area payment scheme	FLATO	Regionalized decoupled flat-rate area payment, different for arable land (area under potato, vegetables and perennial crops excluded) and for permanent grassland. Values estimated: <ul style="list-style-type: none"><li>• 289 € per ha of arable land</li><li>• 243 € per ha of permanent grassland.</li></ul>
Supplemented flat-rate area payment scheme	FLAT1	Regionalized decoupled flat-rate area payment, different for arable land (estimated 235 €/ha) and for permanent grassland (estimated 198 €/ha), supplemented by coupled standard DP scheme measures: <ul style="list-style-type: none"><li>• 100% suckler cow premium and 40% of slaughter premium.</li></ul>

## RESULTS

### Aggregate income effects

With respect to the baseline situation, income situation of analyzed households is estimated to improve in case of all policy scenarios (Table 2). Aggregate model results indicate that total income could on average increase by 3 to 7% and income from agriculture by 9 to 18%

compared to base year 2001. In aggregate, standard DP policy scheme (EUo) is estimated as most income favourable, followed by basic flat-rate area payment option (FLAT0). Average total DP amount received by sample households is estimated to (almost) double, compared to base year 2001. Thus the inflow from direct payments could compensate the effects of the expected decrease of overall producer price level after the accession.

Table 2: Aggregate income effects of alternative DP schemes

Preglednica 2: Učinki različnih schem neposrednih plačil na dohodkovni položaj kmečkih gospodarstev (celotni vzorec)

	Unit	2001	Scenario			
	1000 EUR	2.2	EUo	SAPS	FLAT0	FLAT1
<b>Budgetary support (BS)</b>						
Index 2001 =100	%	100	210.8	186.2	192.6	180.4
Share of direct payments in BS	%	77.4	69.7	65.7	66.8	64.6
Share of LFA payments in BS	%	14.9	16.3	18.4	17.8	19.0
Share of environmental program payments in BS	%	7.0	14.0	15.8	15.3	16.3
<b>Income from agriculture (IA)</b>	1000 EUR	7.4	8.7	8.1	8.2	8.0
Index 2001=100	%	100	117.5	110.2	112.1	108.5
Share of BS in IA	%	29.8	53.5	50.4	51.3	49.6
<b>Total income of agricultural household (TI)</b>	1000 EUR	19.8	21.1	20.5	20.7	20.4
Index 2001=100	%	100	106.5	103.8	104.5	103.2
Share of IA in TI	%	37.3	41.1	39.6	40.0	39.2
Average producer prices						
Index 2001=100	%	100	95.0	95.0	95.0	95.0
Average size of sample agr. households (2001):						
UAA	ha			11.3		
Number of animals	*LU			14.3		
"Real" economic size, calculated from 2001						
total gross margin from agriculture	**rESU			10.6		

\*LU – livestock units; \*\*1 rESU – 1200 euros of total gross margin from agriculture

The main reason why the investigated households could benefit the most in case of EUo scenario could lie in specific structure and high intensity of their production, which both markedly differ from national average.\* On average beef and milk production, favoured under standard DP scheme, contributed almost 50% of total value of agricultural production in base year 2001 (national average according to Rednak (2003) only 39% in the same year) and a half of the average total DP amount received by sample households (EUo scenario).

### Income effects by employment types of agricultural households

Model results analyzed by employment types suggest that income situation is likely to improve after accession for all analyzed employment types compared to base year situation (Table 3). Comparing different policy scenarios all types could benefit the most in case of adoption of EUo scenario, followed by FLAT0 policy option.

\* Sample average in 2001: 11.3 ha of UAA and 14.3 LU (national average according to SORS (2002): 5.3 ha of UAA and 5.7 LU).

Table 3. Income effects of DP policy alternatives by employment types

Preglednica 3. Učinki različnih shem neposrednih plačil na dohodkovni položaj kmečkih gospodarstev po zaposlitvenih tipih

Employment type of agricultural households	Scenario	BS		Share of BS in IA %	TI Index	Share of IA in TI %
		Index	IA			
		2001 = 100 (1000 EUR)	2001 = 100 (1000 EUR)			
Full-time	2001	100 (3.37)	100 (14.10)	23.9	100 (20.79)	67.8
	EUo	216.9	112.1	46.3	108.2	70.2
	SAPS	180.3	103.3	41.7	102.3	68.5
	FLATO	191.1	105.9	43.2	104.0	69.0
	FLAT1	172.1	101.4	40.6	100.9	68.1
Part-time	2001	100 (1.78)	100 (5.26)	33.8	100 (18.14)	29.0
	EUo	238.6	129.6	62.2	108.6	34.6
	SAPS	211.6	120.4	59.3	105.9	33.0
	FLATO	217.1	122.3	59.9	106.5	33.3
	FLAT1	208.9	119.5	59.0	105.7	32.8
Self-employed	2001	100 (3.17)	100 (10.31)	30.8	100 (26.19)	39.4
	EUo	172.8	111.7	47.6	104.6	42.0
	SAPS	163.5	108.8	46.2	103.5	41.4
	FLATO	165.1	109.3	46.5	103.7	41.5
	FLAT1	158.6	107.3	45.5	102.9	41.0
Non-agricultural	2001	100 (0.31)	100 (-1.35)	/	100 (14.96)	/
	EUo	159.3	/	/	100.9	/
	SAPS	199.3	/	/	101.7	/
	FLATO	198.3	/	/	101.7	/
	FLAT1	182.6	/	/	101.4	/
Full-time:	n = 31	UAA = 17.8 ha		LU = 26.7	rESU = 17.9	
Part-time:	n = 47	UAA = 9.8 ha		LU = 12.0	rESU = 8.2	
Self-employed:	n = 22	UAA = 14.3 ha		LU = 14.4	rESU = 14.0	
Non-agricultural:	n = 20	UAA = 1.8 ha		LU = 0.5	rESU = 0.9	

Legend (also for Table 4): BS – Budgetary support, TI – Total income of an agricultural household, IA – Income from agriculture, 1 rESU – 1200 euros of total gross margin from agriculture, / – Not computable

In absolute terms the income effects are estimated as most beneficial to full-time farms (all scenarios). In relative terms however, income impacts are higher on part-time farms and self-employed agricultural households (except in case of EUo scenario). This could be explained by specific production structure and higher production intensity of full-time farms. Milk production prevails in their production structure (it contributed around 40% of value of agricultural production in 2001). For milk sector we can expect a significant price decrease after the accession which could lead to income decreases. Additionally, due to high farming intensity full-time farms are on average assumed not eligible for environmental payments, which together with other rural development payments most significantly improve post-accession income situation of production and more extensive types in terms of input use, especially part-time farms (Kožar *et al.*, 2003).

In relative terms model results reveal a marked redistribution of DP funds to the households that are in terms of production and factor use less intensive (part-time farms). This could be induced by relatively higher share of beef production (contributed around third of value of

agricultural production in 2001) compared to full-time farms and by their lower production intensity, enabling them to participate in rural development programs.

Table 4. Income effects of DP policy alternatives by total income quintiles  
 Preglednica 4. Učinki različnih shem neposrednih plačil na dohodkovni položaj kmečkih gospodarstev po dohodkovnih skupinah

Total income quintile (households ranked by TI in 2001)	Scenario	BS	IA	Share of BS in IA	TI	Share of IA in TI
			Index			
			2001 = 100 (1000 EUR)	2001 = 100 (1000 EUR)	Index 2001 = 100 (1000 EUR)	%
1 <sup>st</sup> quintile (max. 10 946 EUR)	2001	100 (1.62)	100 (1.36)	119.1	100 (8.30)	16.4
	EUo	209.7	197.8	126.3	116.0	27.9
	SAPS	183.9	167.1	131.1	111.0	24.6
	FLAT0	193.0	178.0	129.2	112.8	25.8
	FLAT1	182.9	165.9	131.3	110.8	24.5
2 <sup>nd</sup> quintile (10 946 – 14 896 EUR)	2001	100 (1.38)	100 (2.45)	56.2	100	18.7
	EUo	228.7	150.0	85.7	109.3	25.6
	SAPS	211.5	140.3	84.7	107.5	24.4
	FLAT0	220.6	145.4	85.2	108.5	25.0
	FLAT1	206.7	137.6	84.4	107.0	24.0
3 <sup>rd</sup> quintile (14 896 – 19 782 EUR)	2001	100 (1.49)	100 (3.74)	39.9	100	21.8
	EUo	224.7	131.9	68.0	106.9	26.9
	SAPS	177.5	113.0	62.7	102.8	23.9
	FLAT0	185.7	116.3	63.7	103.6	24.5
	FLAT1	174.6	111.9	62.3	102.6	23.8
4 <sup>th</sup> quintile (19 782 – 26 325 EUR)	2001	100 (2.47)	100 (10.03)	24.6	100	45.0
	EUo	232.9	117.0	49.0	107.7	48.9
	SAPS	206.5	110.6	46.0	104.8	47.5
	FLAT0	213.5	112.3	46.8	105.5	47.9
	FLAT1	198.3	108.5	45.0	103.8	47.0
5 <sup>th</sup> quintile (more than 26 325 EUR)	2001	100 (4.04)	100 (19.26)	21.0	100	50.7
	EUo	186.4	105.2	37.2	102.6	52.0
	SAPS	169.3	101.6	34.9	100.8	51.1
	FLAT0	172.8	102.4	35.4	101.2	51.3
	FLAT1	161.7	100.0	33.9	100.0	50.7
1 <sup>st</sup> quintile:	n = 24	UAA = 7.9 ha		LU = 7.6		rESU = 5.1
2 <sup>nd</sup> quintile:	n = 24	UAA = 7.7 ha		LU = 8.2		rESU = 5.0
3 <sup>rd</sup> quintile:	n = 24	UAA = 7.9 ha		LU = 10.9		rESU = 6.9
4 <sup>th</sup> quintile:	n = 24	UAA = 13.2 ha		LU = 17.4		rESU = 13.1
5 <sup>th</sup> quintile:	n = 24	UAA = 19.9 ha		LU = 27.6		rESU = 22.7

### Income effects by total income groups

Income situation of all groups (quintiles) of households, ranked by total income in year 2001, is likely to improve after the EU accession. In relative terms the income from agriculture and total income could most markedly increase for lower income groups, i.e. Q1 and Q2, whereas the relative increase for highest income group Q5 would be modest (in absolute figures this group, including production more intensive households, would benefit the most compared to other

groups). Further analyses reveal reasons for modest income effects for group Q5. This result is probably due to specific production structure (high share of beef production; around 32% of value of agricultural production in year 2001) and higher intensity in terms of production and factor use. Again, effects of redistribution of DP funds to households more extensive in terms of production and ranked in lower income groups (Q1, Q2) are clearly evident.

## DISCUSSION AND CONCLUSIONS

Post-accession income situation of analyzed agricultural households in Slovenia is estimated to improve in case of all DP policy scenarios on aggregate sample level, as well as considered by employment types and total income groups. Total income results suggest stabilizing effects of inflow from direct payments on expected drop of overall price level after the accession. Tables 2 to 4 reveal that sample agricultural households could benefit more from standard DP policy scheme than from simplified scheme (SAPS) in the immediate post-accession period (2004 to 2006). In case of CAP reform policy options (FLAT0, FLAT1) DP funds could reallocate to households less intensive in terms of production and factor use (part-time farms, lower income groups).

Reasons for redistribution could partially lie in sample characteristics. Sample households are on average, as already mentioned, production and factor more intensive compared to national average (SORS, 2002; Statistical yearbook, 2002). Additionally, sample households significantly differ in structure of production (higher share of milk and beef production) and land use (lower share of permanent grassland)<sup>\*</sup> from national average. Redistribution effects, depicted also in Figure 1, are undoubtedly in line with main CAP reform objectives: decoupling of direct payments, production limitation and multifunctionality enhancement (EU News..., 2003). However, at the same time they could cause delicate structural and income pressures on households that are production<sup>†</sup> and factor more intensive or have higher total income (full-time farms, highest income group).

Considering its stronger income benefits compared to simplified (SAPS) scheme in terms of redistribution of DP funds, standard DP scheme proved to give more acceptable results for the majority of intensive farmers which at the same time represent an important part of farm interest groups. Considering also an additional fact that Slovenian government invested resources to establish a CAP-like DP scheme prior to the EU accession, it is understandable that standard DP scheme (Council Regulation..., 2003) was chosen for the immediate post-accession period.

In respect of CAP reform implementation, model results show that transition to flat-rate area payment options (FLAT0 or FLAT1) could be riskful in terms of redistribution of DP funds. A direct switch to basic flat-rate area payment option immediately after the accession would therefore theoretically be the most preferable solution (Erjavec *et al.*, 2004). Considering that Slovenia implemented CAP oriented DP policy prior to EU accession, the switch would deteriorate income situation of the vital portion of agricultural households (intensive, prevailing beef and milk production). At time of submitting the paper, final decision of Slovenia on CAP reform DP policy scheme was not made yet. However, different supplemented flat-rate area payment schemes were analyzed to design the one that would enable the smoothest switch to CAP reform conditions.

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\* Sample average in 2001: permanent grassland represents 44% of total UAA (national average in 2001: 61% of total UAA).

† Full-time farms contributed almost a half to the total value of agricultural products of sample households in 2001 (similarly income group Q5).

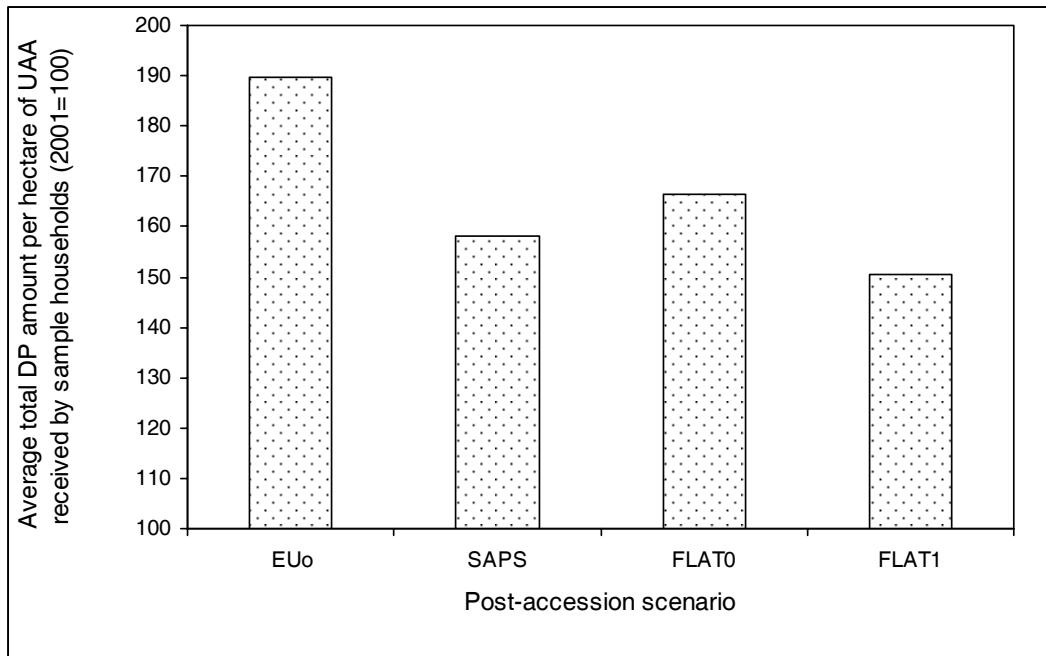


Figure 1. Direct payments per hectare of UAA received by sample households under different post-accession policy scenarios.

Slika 1. Vrednost neposrednih plačil na hektar kmetijskih zemljišč v uporabi, prejetih po različnih popristopnih scenarijih.

Comparison of model results illustrates some general directions of possible impacts of analyzed DP policy options on income situation of sample agricultural households. However, results should be taken with some degree of precaution. Model TIM is recommended to be upgraded in a way to enable modelling of non-agricultural income activities of agricultural households and modelling of additional aspects of DP policy options (Erjavec *et al.*, 2004). Further, database representativeness could be improved. In this respect application of other relevant Slovenian databases, especially IACS database should be taken into consideration.

Finally, different DP policy options should be tested also by applying other empirical tools, which allow a deeper insight into agricultural sector and a more detailed evaluation of other economic effects, especially in the sense of production and structural effects, for example mathematical programming models (Howitt, 1995; Röhm and Dabbert, 2003; Sinabell and Schmid, 2003).

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## EVALUATION OF PUBLIC EXPENDITURE ON ECONOMIC GROWTH OF THE PERIPHERAL SLOVENIA WITH INPUT-OUTPUT MODEL

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### ABSTRACT

Slovenia is witnessing a problem of divergence in economic performance of its regions despite the high importance of the balanced regional development on the policy agenda. After the EU accession the problem of regional disparities is addressed through a wide set of financial mechanisms that affect the regional development; i.e. certain elements of CAP, Structural and Cohesion funds and Community initiatives. The paper is evaluating impacts of public expenditure from these sources on the economic performance of the region Peripheral Slovenia by constructing a regional Input-Output model in the present (2004–06) and the following (2007–13) financial perspective. Results show that the analysed funds can stimulate a notable economic growth of the Peripheral Slovenia especially in the following financial perspective. However; comparisons of the output growth at the national level reveal likely lagging of the region. This means that the anticipated increase of regional development disparities in Slovenia would continue in the future.

Key words: economics / Slovenia / regional development / EU accession / regional input-output model

## OVREDNOTENJE JAVNIH TRANSFEROV NA GOSPODARSKO RAST V PERIFERNI SLOVENIJI Z MODELOM INPUT-OUTPUT

### IZVLEČEK

Slovenija se sooča z razlikami v gospodarski razvitosti med regijami, kljub deklarativeno izraženem pomenu, ki ga uravnoteženemu regionalnemu razvoju posveča politika. Po pristopu k EU naj bi k zmanjševanju regionalnih razvojnih razlik pripomogli finančni mehanizmi Skupnosti, ki vplivajo na dinamiko gospodarske rasti regij. Te finančne mehanizme sestavlja nekateri segmenti Skupne kmetijske politike, Strukturni in Kohezijski sklad ter iniciative Skupnosti. V članku so ovrednoteni gospodarski učinki teh finančnih instrumentov v »sintetični« regiji Periferna Slovenija z izgrajenim regionalnim modelom Input-Output za sedanje finančno obdobje (2004–06) in naslednjo finančno perspektivo (2007–13). Rezultati kažejo, da analizirani transferji lahko opazno spodbudijo ekonomsko rast Periferne Slovenije, še posebej v naslednji finančni perspektivi, primerjave z učinki na nacionalni ravni pa napovedujejo zaostajanje regije. To potrjuje pričakovanja o nadalnjem povečevanju regionalnih razlik v Sloveniji.

Ključne besede: gospodarstvo / Slovenija / regionalni razvoj / vstop v EU / regionalni input-output model

## INTRODUCTION

Despite the fact that balanced regional development has been highly ranked on the policy agenda, Slovenia is witnessing a problem of divergence in economic performance of its regions. Differences in economic development have been deepening between regions throughout the period of economic transition (IMAD, 2003). To a great extent driven by the EU-Accession, Slovenia has tackled this problem by putting in place the institutional set-up and by extending the range of policy instruments with a ‘regional scope’.

As it stands at the moment, the territorial scope of EU policies dealing with cohesion, management of natural resources and rural development, which form the bulk of EU expenditure promoting regional development, Slovenia is treated as one single region.

Nevertheless, the paper attempts to unveil the regional impacts of the abovementioned EU policies to the region of *Peripheral Slovenia* (whole Slovenia except the capital with its surroundings), where effects at the national level are used as a comparative benchmark. The reason for choosing the region which occupies almost whole territory of the country lies in the fact that economic disparities are mainly exhibited in the core – periphery manner.

The central part of Slovenia around the capital city is developing much faster, while Peripheral Slovenia is lagging behind. GDP per capita in Peripheral Slovenia is about 13% below the national average (SORS, 2003) and increased competition following the EU accession may cause further negative effects on the regional disparities. Hence, Peripheral Slovenia is expected to receive the bulk of cohesion expenditure after the accession to the EU.\*

The Peripheral region occupies 87.4% of Slovenian territory and provides residence for about 75% of its population. Over the last decade the number of inhabitants in the Peripheral Slovenia has been stagnating, which has resulted in correspondingly stagnant population density. In terms of settlement distribution, the region is characterised by villages and small towns, and only a few mid-size towns that are main generators of economic exchange and entrepreneurship. The share of people living in rural municipalities (62%) is higher than the national one (55%). In the year 2001 the region contributed around 67% of the national GDP. The region’s GDP per capita was lagging behind the national average by 13% and amounted to 63% of the EU average. The taxable earnings per capita in the region have been weaker than national ones for a number of past years – they reached 92% of national average in 2001.

Despite region’s relatively successful economic recovery after the transition shock, the divergence in the level of economic growth compared to the capital persist. This can be attributed to various reasons, e.g. less favourable sectoral structure (additionally impaired by harsh market conditions), uncompetitive firm structure, emigration and consecutive languishment of human capital (Strategija..., 2002). There can still be found highly agriculture-dependent or declining industrial areas with the lack of working potential and low educational level of population.

The paper is organised as follows. It starts with a brief theoretical discourse on the methodological approach used; namely regional Input-Output (I-O) model. This is followed by a presentation of the approach towards the analysis – the scenario formulation and composition of the vector of the final demand changes – the central step in the I-O simulation. The paper ends with commenting some of the most straightforward results and by discussing the implications for further research.

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\* The process of regionalisation in Slovenia is under way at the moment and there are various concepts at stake. The concept used in our analysis is one of them and does not prejudice the likely regional division. As a matter of fact, according to the implications of the EU cohesion policy in the next programming period (2007–2013), Slovenia will most likely be treated as one region.

## METODOLOGY AND DATA

A wide array of analytical tools has been developed for the purpose of quantitative economic evaluation of public expenditures. One of the well established classes of modelling approaches used is based on the input-output paradigm developed by Leontief and empirically applied for more than half of the century (Sadoulet and de Janvry, 1995).

With the development of more capable modelling tools (e.g. Computable General Equilibrium Model, econometric short-term forecasting macro-models) relevance of the linear deterministic models has certainly decreased, however, for the analysis at the regional level the interest for input-output technique is significantly increasing (Armstrong and Taylor, 2000). The main reason for popularity is robustness of the technique that can be implemented empirically despite data shortages (Thirlwall, 2003).

In the paper a regional I-O model was constructed to evaluate economic effects of the EU funds anticipated in the periods 2004–06 and 2007–13. Theoretical features of the methodology used are presented in the following sub-sections.

### Input-Output modelling

Economic policies might cause structural changes that are facilitated by complex interactions among the economic sectors and agents (Intriligator, 1983), therefore, for evaluation of public expenditure an analytical approach is preferred that effectively estimate sectoral interdependence (Sadoulet and de Janvry, 1995). One of the widely applied theoretical paradigms for analysing structural change is I-O analysis (Thirlwall, 2003). The technique is also adequate for the evaluation of economic effects of public expenditures that are initiating changes of the final demand (Richardson, 1972).

I-O techniques numerically model the relationships among the productive sectors of an economic system. By showing details of the flow of goods and services among industries, they describe the process of production, the use of goods and services, and the income generated in production (O'Connor and Henry, 1975).

The starting point of an I-O model is the assumption that the quantity of the product used as an input of a sector ( $X_{ij}$ ) is in proportion to the total output of this sector ( $X_j$ ):

$$X_{ij} = a_{ij} X_j \quad [1]$$

The quantity of a product required by the sector for production of one unit of output ( $a_{ij}$ ) is determined by the technical coefficient. They define the effect of production increase of a sector on the input demand.

Demand and supply in an I-O system are assumed to be in equilibrium hence applying the equation [1] can now be written:

$$X_i = \sum_{j=1}^n a_{ij} X_j + F_i \quad [2]$$

Total supply of a product ( $X_i$ ) is equal to the sum of intermediate demand ( $\sum_{j=1}^n a_{ij} X_j$ ) and final demand ( $F_i$ ) for this product.

From a series of equations like [2] representing all sectors of the analysed economy technical coefficients can be derived and with applying matrix algebra the effects of demand change for one product on the output of all other sectors of economy can be quantified.

This system of equation can be written in the matrix form as:

$$\mathbf{X} = \mathbf{AX} + \mathbf{F} . \quad [3]$$

and the inverse of the equation [3] is:

$$\mathbf{X} = (\mathbf{I} - \mathbf{A})^{-1} \mathbf{F} , \quad [4]$$

where  $(\mathbf{I} - \mathbf{A})^{-1}$  represents the total multipliers matrix – known as *Leontief* inverse.

The basic technique of the I-O modelling is application of the total multipliers matrix for calculating total input requirements for a unit value of final demand. However, the change in final demand does not affect only the direct requirement in the production process of the analyzed sector itself, but also all indirect requirements resulting from intermediate product deliveries from other sectors. I-O method therefore includes both direct and indirect input necessary to satisfy change of final demand (Thirlwall, 2003; Miller and Blair, 1984).

From the times of its foundation the I-O technique had remained rather simple in comparisons with other contemporary models since it is subject to some restrictive assumptions:

- each sector's demand or intermediate inputs changes in direct proportion to output from that sector;
- the models are final demand driven;
- no technological change occurs;
- there is no substitution of intermediate inputs;
- different production activities can be grouped into homogeneous sectors, each producing one product (Rose and Miernyk, 1989).

Empirical limitations of the method arise from these assumptions; however; the technique is well capable to study elementary intersectoral relations and directions of potential effect from changed final demand in the economy (Sadoulet and de Janvry, 1995). Further, in recent years, computable general equilibrium (CGE) and social accounting matrices (SAM) represent newer paradigms based on the I-O logic that offer solutions to some of the inherent problems found in primary I-O analysis (Rose and Miernyk, 1989; Thirlwall, 2003).

## **Regional I-O models**

Applications of the I-O methodology initially concerned national economies, however, soon after their affirmation, attempts to use it for the purposes of regional analysis evolved. First of them is the work of Isard and Kuene (1953). Miernyk (1982) provides detailed bibliographic presentation of the most important studies dealing with the construction and specification of regional input-output models.

The central task in the regionalisation procedure is adaptation of a national I-O model in order to reflect particular features of the regional economy. A regional I-O model can be constructed either by using primary data for the regional intersectoral transactions (survey method) or, more frequently, by applying various techniques of regionalizing the national I-O model (non-survey method). With the non-survey methods derivation is based on various secondary sources of statistical data applied on the national model (Richardson, 1972). Several approaches were proposed however, after the development of so called hybrid regionalisation, popularity of other approaches was reduced (Rose, 1982). Hybrid techniques combine the specific data and information from small scale surveys with the existing national table.

For the purpose of regionalisation of the national I-O table in the paper the GRIT technique was applied. The method was proposed by Jensen *et al.*, (1979). GRIT is a formalized non-survey regionalisation method with facility to insert survey data at any stage of the compilation

procedure. The main motivation for the methodology selection was lack of primary regional data. The regionalisation procedure is presented in the following subsection.

### Derivation of the regional I-O table for Peripheral Slovenia

The basic source of data for regionalisation was the national 59 sector input-output table estimated by Slovenian Statistical Office for the year 2000 (SORS, 2003). Additionally some secondary data was used: employment at the national and regional level (SORS, 2004); structure of the national and regional value added (SORS, 2004); distribution of the income tax base (IMAD, 2004) and superior data on agricultural sector (SORS, 2004).

Decision about the appropriate sectoral disaggregation was adopted by taking into account the structure of economic activities in the analysed region. Due to relative size of the regional economy (it accounts for about two thirds of the national GDP), the region exhibits a great diversity of economic activities. Somewhat specific pattern of regional development in Slovenia before the transition\*, together with the relative size of the region in national economy were the main arguments to analyse a relatively broad set of economic activities. Thus, the final regional input-output table comprises of 29 sectors.

A starting point for regionalisation was the adjustment to the national I-O table with total flows. The national flows matrix was converted to a technical coefficient matrix as follows:

$$A = Z \hat{X}^{-1} \quad [5]$$

where A represents the matrix of technical coefficients, Z matrix of intersectoral transaction flows and  $X^{-1}$  the inverse of diagonal output matrix derived from output vector.

In the stage of the adjustment for regional technical coefficients, a non-survey method of Simple Location Quotients (SLQ) was used as follows.

$$A^R = \hat{q} A^N \quad [6]$$

Regional technical coefficients are denoted by subscript R and national ones by N. The SLQ vector is denoted by q and they were derived from the relevant secondary statistical data (e.g. breakdown of employment data by sectors, E). Simple location quotient for sector  $i$  can therefore be calculated:

$$q_i = \frac{E_i^R / E^R}{E_i^N / E^N} \quad [7]$$

The method used assumes that sectors whose relative importance at a regional level is equal or greater than at a national level ( $q_i \geq 1$ ) are able to satisfy intermediate demand within the region and coefficients therefore remain the same as the national ones. Otherwise, the sector is supposed not to be self-sufficient and the corresponding national coefficient is multiplied by  $q_i$ .

In the next stage aggregation of the sectors has been conducted, hence first the regional matrix of technical coefficients was modified as follows:

$$A_{(1)}^R = A_{(0)}^R \hat{w} \quad [8]$$

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\* This pattern of regional development is a consequence of the so called ‘poli-centric’ approach towards regional development policy. It is characterised by intensive (sometimes forced) public intervention in order to assure a spatially scattered and diversified industry mix (Nared, 2003).

The original technical coefficients were adjusted by the vector of employment weights  $w$ , by which approximation towards the regional structure of economic activities is made.

The next step is derivation of a prototype transactions table with an estimation of regional output. These estimates were determined by using employment ratios.

$$X_i^R = X_i^N \frac{E_i^R}{E_i^N} \quad [9]$$

The next step in the prototype table derivation was the estimation of three components of final demand. The household consumption was calibrated by the share of regional income tax base in the total income tax base.

The remaining two components of the final demand, namely exports and other final demand categories (comprised of government expenditures, gross capital formation, expenditures by non-profit institutions and changes in inventories) were estimated simultaneously with balancing of intermediate consumption. The starting values were derived from the national tables and later adjusted downwards using employment and location quotient. All elements within the transaction matrix were treated equally and thus reduce the on or off-diagonal elements according to the value of output, final demand, share of imports in every cell of the national table and expert knowledge. Intermediate consumption rows of 12 sectors were reduced and 16 on-diagonal elements of primary and secondary sectors were reduced as well.

In the final checks and balancing stage some inconsistencies and errors were discovered and corrected. Finally, the balanced input-output table was composed which was believed to result in the realistic regional multipliers.

### **Policy scenario definition**

The impact analysis using the constructed I-O model has been carried out in two sets of scenarios with respect of the financial programming period. The first set of scenarios includes the policy instruments available in the pre-accession period and in the first programming period after the EU accession (2004–06), whereas the second set of scenarios takes into account the proposed EU budgetary appropriations for the new financial perspective 2007–13.

Description of policy instruments and the corresponding financial breakdown for the period 2004–2006 was derived from various national and EU programming documents (Single programming document, Cohesion strategy, Rural development plan). In the case of CAP expenditure from the Guarantee section, where allocation of funds is not subject to programming, estimates proposed out by the Agricultural Institute of Slovenia were used.

For the financial perspective 2007–2013 budgetary appropriations were used as outlined in the Communication from the European Commission (COM/2004/487). The document only describes the overall financial framework by expenditure headings, while appropriations for commitments by Member States are not yet presented (December 2004). Qualified estimates of EU budgetary appropriations were obtained in consultation with the corresponding national working documents for the fields of cohesion (Mrak and Rant, 2004) and agricultural budget expenditure (MAFF, 2004).

In the New Financial Perspective 2007–2013 status of Slovenia in terms of its eligibility for EU cohesion expenditure is still not ultimately determined, therefore two options of expected budgetary appropriations were proposed. "Conservative" estimates of EU budgetary inflows relate to the less favourable status of Slovenia. This entails full eligibility for the Cohesion fund support, whereas in the case of the Structural funds, Slovenia is assumed to be treated as a 'phasing in' region within the objective 'Regional competitiveness and employment'. More optimistic estimates differ only in the eligibility status for structural funds. They derive from an

assumption that Slovenia will remain eligible for higher rates of structural fund support within the “Convergence objective”, although this status will be only transitional due to expected statistical effects.

Once the national budgetary appropriations were consolidated the annuities needed to be regionalised. The funds attributed to *Peripheral Slovenia* have been estimated according to the selected regionalization weights: share of active population in the region, GDP contribution of the region, share of population in the region, ESU share of the region. These were applied according to the characteristics of each policy instrument.

Envisaged public expenditure for the *Peripheral Slovenia* for the two studied budgetary periods by the main policy instruments is presented in the table 1.

Table 1. National and EU funds available for the Peripheral Slovenia in the periods 2004–2006 and 2007–2013 (annuities, in million Slovenian Tolars – prices 2000)

Preglednica 1. Domača in EU proračunska sredstva namenjena regiji 'Periferna Slovenija' v programskeh obdobjih 2004–2006 in 2007–2013 (vrednosti na letni ravnini, v milijonih Slovenskih tolarjev, stalne cene za leto 2000)

Policy instrument	Period 2004–2006	Policy instrument	Period 2007–2013	
			“Phasing out”	“Phasing in”
SAPARD	2 200.2	Structural funds	36 857.8	13 425.2
ISPA	8 136.6	ERDF-type measures	23 363.0	8 509.8
Structural funds	14 811.1	ESF-type measures	13 494.8	4 915.4
ERDF	7 644.5	Cohesion fund	33 292.7	33 500.0
ESF	4 441.7	Territorial Integration	6 301.6	4 248.2
EAGGF	2 614.8	EAGGF – direct payments	22 687.1	22 687.1
FIFG	110.0	Agricultural Rural development fund	6 085.1	6 085.1
Cohesion fund	8 109.6	CAP RD-Guarantee type	25 967.3	25 967.3
Interreg	1 945.1	CAP RD-Guidance type	2 793.5	2 793.5
Equal	376.3	Fisheries	227.6	227.6
Schengen	6 496.6			
EAGGF – direct payments	17 829.2			
EAGGF Guarantee – rural development	17 339.8			

Source: Own compilation based on various national and EU sources

To evaluate the economic impacts of the formulated budgetary appropriations with the constructed I-O model the funds had to be distributed according to the expected effects they will have on the final demand for the economy's outputs. This external shock is aggregated within the vector of final demand changes. The structure of investment demand from the national I-O table (year 2000) was taken as a basis for the distribution of funds.

No additional weights or corrections were applied for assessing the structure of demand in the case of policies with general ‘investment’ patterns, whereas “objective-oriented policies” were treated specifically. Allocation of funds along the vector of final demand has been determined in accordance with the scope and “mechanism” of the policy as outlined in the programming documents. In the case of policies with an income support character (e.g. decoupled direct payments in agriculture), the effects were distributed according to the household final demand structure.

In the last stage of the vector definition the origin of demanded good has been taken into account. In the case of investment demand the share of domestic goods in investment from the

national I-O table was applied, whereas for the correction of the household final demand the proportions of the domestic supply in total market supply was used.

In total five scenarios were formulated for both programming periods:

Scenario 1: “pre-accession support”:

In this scenario is assumed that only SAPARD and ISPA funds are available beside the existing national policies. In order to estimate the maximum potential of the pre-accession support, we have decided for a (rather unrealistic) assumption of a 100% absorption rate of these funds. This scenario was applied only for the first programming period.

Scenario 2: “partial integration”:

This scenario attempts to give the “conservative” estimates of various EU financial mechanisms after the accession to the EU. In the first programming period after the accession, these relate to a lower absorption level of the funds.\* For the CAP-related expenditure the proposed absorption is 85% in the case of direct payments and 70% absorption rate for rural development measures financed by EAGGF-Guarantee part. The assumed absorption of Structural funds and Cohesion fund is 50%, whereas the rate for Schengen facilities assistance is assumed to be somewhat higher (70%). Regarding the structural funds allocation in scenario 2 Slovenia is treated as the “phasing in” region.

In the attempt to model CAP direct payments as being fully decoupled the total amount is transferred to the final demand of households. This approach was used as a proxy illustrating the final structure of decoupled direct support in agriculture.

Scenario 2a: “partial integration DP coupled”:

In this sub-version of the scenario 2 the attempt is made to evaluate the different treatment of the CAP direct payments. It is assumed that if the direct payments are fully coupled the entire sum is being spent according the vector of intermediate demand of the agricultural sector.

Scenario 3: “full integration”:

In contrast to the scenario 2, this scenario attempts to provide the information about the maximum potential effect of the analysed funds. In this respect, we have taken an optimistic assumption that all available funds will be absorbed (100% absorption). This entails also favourable status within the Structural funds distribution, where Slovenia is assumed to be treated as the “phasing out” region. Direct payments are assumed to be fully decoupled.

Scenario 3a: “full integration DP coupled”:

This sub-scenario rests on same assumptions as the scenario 3, with the only exception that CAP direct payments are, similarly as in scenario 2a, assumed to be fully coupled with production.

Four separate runs of each scenario were carried out. Firstly two runs were made for each programming period, except for the scenario 1 which is not relevant for the period 2007–13. In order to provide a benchmark for assessment of regional impacts, the policy scenarios were tested also at the national level with the national I-O model and appropriate budgetary outlays.

## RESULTS

### Change in the gross output

The most straightforward output of scenario analysis with the I-O model is the change in gross output by sectors. Main results presenting the percentage change of the gross output in

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\* Experience gained from previous enlargements (European Commission 2002, 2003, 2004) and recent experience with low absorption levels of the pre-accession funds in Slovenia (DAAC Consortium, 2004)), have led us to believe that in the initial period after accession part of the allocated funds may remain unused.

comparison to the base year (2000) for the main sectoral aggregates (agriculture, industry, services) are presented in Table 2.

Due to a relatively limited change of final demand caused by ISPA and SAPARD programmes in Slovenia, no considerable economic impacts were detected as a consequence of the pre-accession programmes (Scenario 1). This has happened even under the assumption of 100% absorption level. No conclusive evidence is given. As a matter of fact, only the construction sector exhibits noticeable (2%) increase of gross output mainly due to large-scale infrastructure investment projects supported by ISPA.

Taking into account more pessimistic estimates of EU budgetary inflows (Scenario 2), the projected increase of overall regional output is 1.1% in 2004–06 and 2.6% in 2007–13 period. Estimated effects under the Scenario 2 are higher at the regional level in comparison to the national figures for about one tenth in the period 2004–06 and 14% in the period 2007–13! Impacts on agriculture are projected to surpass the aggregate figure in 2004–06 in both territorial scopes. In contrast, it is expected to experience a slight lag behind the average levels of output increase in the period 2007–13. No significant implications are expected in the manufacturing sector, where only sectors of mining and quarrying, food manufacturing, supply of electricity, water and gas surpass the average levels of output increase in the period 2004–06. In 2007–13, favourable output increase prospects diminish in the sectors of food manufacturing and supply of electricity, water and gas, whereas significant improvements are projected for production of other non-metallic mineral products. The highest increases are again anticipated in the construction sector, whose output as a consequence of EU public expenditure is projected to grow by 2.3% in 2004–06 and by 10.0% in 2007–13. Most of this is due to infrastructural investments, and partly also due to investments in real estate (which form a significant part of Structural fund support) and transitional support for Schengen facilities in 2004–06.

Table 2. Simulation results: percentage changes in total output by analysed policy scenarios

Preglednica 2. Rezultati simulacijskih izračunov: odstotne spremembe v agregatnem outputu po obravnavanih scenarijih

	Scenario 1		Scenario 2		Scenario 2a		Scenario 3		Scenario 3a		
	Unit	mio. SIT	%	mio. SIT	%	mio. SIT	%	mio. SIT	%	mio. SIT	%
<b>Peripheral Slovenia, 2004–2006</b>											
Agriculture		227	0.11%	2 416	1.17%	3 121	1.52%	3 204	1.56%	4 077	1.98%
Industry		14 322	0.43%	25 749	0.77%	32 246	0.97%	42 912	1.29%	50 954	1.53%
- of which construction		10 953	1.99%	12 412	2.25%	20 078	3.64%	23 266	4.22%	32 753	5.94%
Services		2 545	0.10%	36 334	1.40%	25 760	1.00%	50 847	1.97%	37 760	1.46%
<b>Total</b>		<b>17 094</b>	<b>0.28%</b>	<b>64 498</b>	<b>1.05%</b>	<b>61 127</b>	<b>1.00%</b>	<b>96 963</b>	<b>1.58%</b>	<b>92 791</b>	<b>1.51%</b>
<b>Slovenia, 2004–2006</b>											
Agriculture		221	0.09%	2 877	1.22%	3 679	1.57%	3 809	1.62%	4 802	2.04%
Industry		14 490	0.33%	32 307	0.75%	39 343	0.91%	54 074	1.25%	62 782	1.45%
- of which construction		11 183	1.39%	15 853	1.97%	24 403	3.03%	29 861	3.71%	40 442	5.02%
Services		3 084	0.08%	47 262	1.16%	35 038	0.86%	66 667	1.64%	51 539	1.27%
<b>Total</b>		<b>17 796</b>	<b>0.21%</b>	<b>82 446</b>	<b>0.96%</b>	<b>78 060</b>	<b>0.91%</b>	<b>124 551</b>	<b>1.44%</b>	<b>119 123</b>	<b>1.38%</b>
<b>Peripheral Slovenia, 2007–2013</b>											
Agriculture		-	4 696	2.28%	5 807	2.82%	5 114	2.49%	6 225	3.03%	
Industry		-	88 657	2.66%	98 889	2.97%	110 126	3.30%	120 359	3.61%	
- of which construction		-	55 310	10.03%	67 382	12.21%	70 112	12.71%	82 185	14.90%	
Services		-	67 521	2.61%	50 869	1.97%	86 556	3.35%	69 904	2.70%	
<b>Total</b>		-	160 874	<b>2.63%</b>	155 565	<b>2.54%</b>	201 797	<b>3.29%</b>	196 488	<b>3.21%</b>	
<b>Slovenia, 2007–2013</b>											
Agriculture		-	4 850	2.06%	5 985	2.55%	5 379	2.29%	6 513	2.77%	
Industry		-	111 891	2.58%	121 841	2.81%	139 817	3.23%	149 767	3.46%	
- of which construction		-	73 051	9.07%	85 142	10.57%	92 361	11.47%	104 452	12.97%	
Services		-	81 248	2.00%	63 960	1.58%	108 693	2.68%	91 405	2.25%	
<b>Total</b>		-	197 989	<b>2.30%</b>	191 786	<b>2.22%</b>	253 888	<b>2.94%</b>	247 686	<b>2.87%</b>	

Model estimates for service sector in total reveal 1.4% increase in 2004–06 and 2.6% by 2007–13 which is above the average. The most positive prospects are projected for wholesaling, tourism and real estate, renting and business activities. The model results however suggest no substantial role of EU funds for improvements in activities dealing with human capital in the period 2004–06. The situation is likely to improve by 2007–13, where EU funds are likely to yield higher output increase (3.0%) in education.

Above described trends in projected output increase for individual sectors are not significantly changed if direct payments in agriculture are assumed to be fully coupled (Scenario 2a). In general, levels of output increase are somewhat lower (5%), apart from the sectors of agriculture and construction. The projected output increase in agriculture in the region would be higher by almost one third in 2004–06 and around one quarter in 2007–13 if direct payments are fully coupled. Implications of reinvestment of public transfers to agricultural production are therefore significant.

Scenarios 3 and 3a provide a benchmark for potential maximum impact of the analysed public funds. If this "optimistic" scenario of EU budgetary inflows was realised, the gross regional output would increase by more than 1.5% for the period 2004–06, whereas the corresponding levels of aggregate output increase in period 2007–13 would be even higher, i.e. 3.3% for Scenario 3 and 3.2% for Scenario 3a. Rather comparable trends concerning output increase for sectors can be observed as in the previous two scenarios (2 and 2a).

Additional insight into the "nature" of modelled public transfers is obtained through the presentation of sectoral distribution of the total effects in Table 3. It is confirmed, that there are no major differences in the structure of the effects from the national-regional comparisons; however, impact on agriculture and industry tends to be somewhat higher in the Peripheral Slovenia, whereas results are more favourable for services. Taking into account the structure of economic activities in the region studied this result is not surprising.

Table 3. Sectoral contribution to the total effects of the public expenditure by analysed policy scenarios

Preglednica 3. Delež posameznih sektorjev v agregatnem učinku javnofinančne porabe po obravnavanih scenarijih

	Scenario 1	Scenario 2	Scenario 2a	Scenario 3	Scenario 3a
<b>Peripheral Slovenia, 2004–2006</b>					
Agriculture	1.33%	3.75%	5.11%	3.30%	4.39%
Industry	83.79%	39.92%	52.75%	44.26%	54.91%
- of which construction	64.07%	19.24%	32.85%	23.99%	35.30%
Services	14.89%	56.33%	42.14%	52.44%	40.69%
<b>Total</b>	100.00%	100.00%	100.00%	100.00%	100.00%
<b>Slovenia, 2004–2006</b>					
Agriculture	1.24%	3.49%	4.71%	3.06%	4.03%
Industry	81.43%	39.19%	50.40%	43.42%	52.70%
- of which construction	62.84%	19.23%	31.26%	23.97%	33.95%
Services	17.33%	57.32%	44.89%	53.53%	43.27%
<b>Total</b>	100.00%	100.00%	100.00%	100.00%	100.00%
	Scenario 2	Scenario 2a	Scenario 3	Scenario 3a	
<b>Peripheral Slovenia, 2007–2013</b>					
Agriculture	2.92%	3.73%	2.53%	3.17%	
Industry	55.11%	63.57%	54.57%	61.26%	
- of which construction	34.38%	43.31%	34.74%	41.83%	
Services	41.97%	32.70%	42.89%	35.58%	
<b>Total</b>	100.00%	100.00%	100.00%	100.00%	
<b>Slovenia, 2007–2013</b>					
Agriculture	2.45%	3.12%	2.12%	2.63%	
Industry	56.51%	63.53%	55.07%	60.47%	
- of which construction	36.90%	44.39%	36.38%	42.17%	
Services	41.04%	33.35%	42.81%	36.90%	
<b>Total</b>	100.00%	100.00%	100.00%	100.00%	

Comparably, there are no major differences in the structure of the effects between the two analysed programming periods. The results suggest that in the programming period 2007–13 output increase will be slightly more on the side of industry sector, especially construction. This is due to projected proportional increase of funding in the fields of productive and infrastructural investments.

## CONCLUDING REMARKS AND FURTHER RESEARCH

Paper tries to quantify the effects of EU funds on the region of Peripheral Slovenia using the Input–Output model. Analysis about the magnitude and effects distribution of various sources of EU public expenditure was made. Policy relevance of the research undertaken can be argued by provision of a valuable insight into the pattern of policy expenditure through various sectors of the regional economy. The following conclusions can be derived.

The results suggest that the analysed funds can bring a significant contribution to the overall output increase of the regional economy after the accession – especially in the 2007–13 period; whereas this can not be confirmed for the pre-accession funds. In this respect, the significance of pre-accession funds can be seen more in terms of institutional building and preparing of the implementation structures for successful absorption of funds after the accession.

Optimistic scenarios about the accession effects (Scenario 3) for 2004–06 and 2007–13 provide a benchmark or the potential maximum impact of the analysed public funds. If this “optimistic” scenario, assuming full absorption of available funds within the region was realised, the gross regional output would increase by 1.6% for the period 2004–06, whereas the corresponding aggregate increase in 2007–13 would be significantly higher, i.e. 3.3%.

However, the favourable post-accession effects should be regarded with some caution. There are various factors that can aggravate the optimistic view expressed with the “benchmark” results presented by the Scenario 3. These factors range from budgetary (status of Slovenian regions for EU cohesion expenditure in 2007–13, limited co-financing capacities of national budget) to organisational ones (implementation structures, availability of matching private capital, lower absorption level). The abovementioned factors could significantly deteriorate favourable results. Our results suggest that these effects could result in about one third lower growth in total output.

The question whether the analysed funds will reduce regional disparities was tackled by the comparison of the regional and national modelling results. As a general observation, there are no major differences in the structure of effects between Slovenia and Peripheral Slovenia region. In both cases, high public investments are channelled into labour intensive sectors (construction, agriculture) with low labour productivity. Our results also show that the impacts of analysed funds on output are slightly higher in the Peripheral Slovenia, although; the difference is rather moderate. For the actual financial perspective the projected growth in the region is about one tenth higher in comparison to the national average, whereas for the financial period 2007–13 the expected advantage of the region is somewhat higher (14%). Our results therefore suggest that the trend of increasing development disparities in Slovenia is will very likely to be stabilised. The question, however; remains whether the dynamic of convergence is sufficient.

Limitations of the research undertaken also have to be acknowledged. First of all, it has to be borne in mind that financial transfers from the EU budget represent only one dimension of the accession-related effects. The analysis does not deal with other important aspects of integration, such as trade effects and increased competition, division of labour, specialisation and change of relative prices. Limitations of the research arise also from the applied methodology – assumptions of the static I-O model are rather restrictive.

However, provided that both the national and regional I-O model is constructed accurately enough, theoretically implausible assumptions of the model are in some respects overshadowed

by its empirical realism and simplicity. With this in mind we can state that this approach towards modelling of policy expenditures gives at least approximate information about the expected changes in sectoral output.

Another warning goes to the fact that the I-O methodological framework is useful only for measuring "hard" tangible impacts, which therefore inevitably results in their over-valuation against the impacts of "soft" investments. With this methodological approach also no aspects is related to the flow of externalities (e.g. food safety, environmental management, rural development). Since these externalities seems to gaining importance on the policy agenda in the Community, they should not be neglected from the future analysis. Upgrading I-O based models with dynamics features is probably the first step after the exercise presented in this paper. But as for intangible effects, some steps in other dimension will be needed.

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Sprejemamo izvirne znanstvene članke, predhodne objave in raziskovalne notice s področja zootehnike (genetika, mikrobiologija, imunologija, prehrana, fiziologija, ekologija, etologija, mlekarstvo, ekonomika, živalska proizvodnja in predelava živalskih proizvodov, tehnologija in dokumentalistika) v slovenskem in angleškem jeziku, znanstveno pregledne članke samo po poprejnjem dogovoru. Objavljamo tudi prispevke, podane na simpozijih, ki niso bili v celoti objavljeni v zborniku simpozija. Če je prispevek del diplomskega, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Navedbe morajo biti v slovenskem in angleškem jeziku.

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V besedilu navajamo v oklepaju avtorja in leto objave: (priimek, leto). Če sta avtorja dva, pišemo: (priimek in priimek, leto), če je avtorjev več, pišemo: (priimek in sod., leto). Sekundarni vir označimo z »navedeno v« ali »cv.«. Seznam virov je na koncu prispevka, neoštevilčen in v abecednem redu. Vire istega avtorja, objavljene v istem letu, razvrstimo kronološko z a, b, c. Primer: 1997a. Navajanje literature naj bo popolno: pri revijah letnik, leto, številka, strani; pri

knjigah kraj, založba, leto, strani. Za naslove revij je dovoljena uradna okrajšava, za okrajsanimi besedami naj bodo vedno pike. Navedbo zaključimo s piko. Nekaj primerov:

- Fraser, A.F./ Broom, D.M. Farm animal behaviour and welfare. London, Bailliere Tindall, 1990, 437 str.
- Hvelplund, T. Protein evaluation of treated straws. V: Evaluation of straws in ruminant feeding (ur.: Chenost, M./ Reiniger, A.). London, Elsevier Applied Science, 1989, 66–74.
- Stekar, J.M.A. Vsebnost makro elementov v slovenski mrvi. V: Posvetovanje o prehrani domačih živali »Zadravčevi-Erjavčevi dnevi«, Radenci, 1997-10-27/28. Murska Sobota, Živinorejsko-veterinarski zavod za Pomurje, 1997, 105–117.
- Stekar, J.M.A./ Golob, A./ Stibilj, V./ Koman Rajšp, M. Sestava in hranilna vrednost voluminozne krme v letu 1990. Zb. Bioteh. Fak. Univ. Ljublj., Kmet. Živin., 58(1991), 149–155.
- Stekar, J.M.A./ Pen, A. Sadržaj natriuma, cinka i mangana u stočnoj hrani sa travnatih površina. Agrohemija, 21(1980)1–2, 7–15.

## Oddaja

Avtorji prispevke oddajo v dveh izvodih, enega z dvojnim razmikom med vrsticami in največ 35 vrstic na strani, in na disketi. Priložijo tudi izjavo s podpisi vseh avtorjev, da avtorske pravice v celoti odstopajo reviji.

Prispevke recenziramo in lektoriramo. Praviloma pošljemo mnenje prvemu avtorju, po želji lahko tudi drugače. Če urednik ali recenzenti predlagajo spremembe oz. izboljšave, vrne avtor popravljeno besedilo v 10 dneh v dveh izvodih, enega z dvojnim razmikom. Ko prvi avtor vnese še lektorjeve pripombe, odda popravljeno besedilo v enem izvodu in na disketi ter vrne izvod z lektorjevimi popravki.

Prispevke sprejemamo vse leto.

## NOTES FOR AUTHORS

### Papers

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Papers in Slovenian language should have tables, graphs, figures and appendices in both languages, Slovenian language being the first. Titles of graphs and figures are below them. Figures and graphs are part of the text. Clearly marked original figures should be added (photographs or separate graphic files); they can be returned upon request. Latin expressions are written in italics. Decimal coma is used in Slovenian and decimal point in English. Papers in English should contain abstract in Slovenian and *vice versa*.

The papers should be condensed, short and should not exceed 12 pages. Microsoft Word 97 or later version (Windows) should be used, fonts Times New Roman, size 12 in text and tables (in large tables size 10 is allowed), Arial for graphs and figures (letter size at least 9) and Courier for nucleic- and amino acid sequence alignments should be used; right margin 2.0 cm, left margin 2.5 cm; *pagina viva* in one line, size 10, author(s) and abbreviated title of the paper ending with a full stop. Examples: Štuhec, I. and Siard, N. Pig Behaviour. Stibilj, V. *et al.* Determination of fatty acids composition ... milk samples in Slovenia.

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The type of the paper should be indicated on the first page on the right side in Slovenian and English language following by title of the paper and authors. Full names of authors are used (first name and surname). Each name of the author should have been added an index, which is put immediately after the author(s), and contains address of the institution and academic degree of the author, in the language of the paper. The address of the institution in which the author works is indicated. If the research was realised elsewhere, the author should name the headquarters of the institution. E-mail is optional.

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- Fliegerová, K./ Pažoutová, S./ Hodrová, B. Molecular genotyping of rumen fungi based on RFLP analysis. *Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Zooteh.*, 72(1998), 95–98.
- Fraser, A.F./ Broom, D.M. Farm animal behaviour and welfare. London, Bailliere Tindall, 1990, 437 p.
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- Ristič, M./ Klein, F.W. Schlachtkörperwert von Broilern verschiedener Herkunfte. *Mitteilungsblatt der Bundesanstalt fuer Fleischforschung*, Kulmbach, 101(1988), 8045–8051.
- Stekar, J.M.A. Silage effluent and water pollution. In: 6<sup>th</sup> International Symposium "Animal Sciences Days", Portorož, 1998-09-16/18, Slovenia. *Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Supl.*, 30(1998), 321–325.

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