



University of Belgrade, Technical Faculty in Bor



ECOENTER

**30th International Conference Ecological Truth
& Environmental Research
2023**

Proceedings

**Editor
Prof. Dr Snežana Šerbula**





University of Belgrade, Technical Faculty in Bor



ECO-TRUTH

30th International Conference Ecological Truth
& Environmental Research
2023

Proceedings

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PREFACE

The 30th international conference Ecological Truth & Environmental Research – EcoTER'23 kept three areas in focus: ecology, environmental protection and sustainable development. The conference will be held on Mt Stara Planina in hotel Stara Planina, Serbia, 20–23 June 2023. The monograph is published on the occasion of the 30th anniversary of the conference. On behalf of the scientific and organizing committee, it is a great honor and pleasure to wish all the participants a warm welcome to the conference.

The monograph is published on the occasion of the 30th anniversary of the conference.

We hope to convey the message of the conference, which is that a transformation of attitudes and behavior would bring the necessary changes. This is also an opportunity for the participants who are experts in this field to exchange their experiences, expertise and ideas, and also to consider the possibilities for their collaborative research.

The 30th international conference Ecological Truth & Environmental Research – EcoTER'23 is organized by the University of Belgrade, Technical Faculty in Bor, and co-organized by the University of Banja Luka, Faculty of Technology, the University of Montenegro, Faculty of Metallurgy and Technology – Podgorica, the University of Zagreb, Faculty of Metallurgy – Sisak, the University of Pristina, Faculty of Technical Sciences – Kosovska Mitrovica and the Association of Young Researchers, Bor.

These Proceedings 103 papers from the authors coming from the universities, research institutes and industries in 11 countries: Australia, USA, Brazil, Spain, Portugal, Libya, Italy, Bulgaria, Bosnia and Herzegovina, North Macedonia, and Serbia.

As a part of this year's conference, the 5th Student Session – EcoTERS'23 is being held. We appreciate the contribution of the students and their mentors who have also participated in the conference.

The support of the Gold donor and their willingness and ability to cooperate has been of great importance for the success of the EcoTER'23. The organizing committee would like to extend their appreciation and gratitude to the Gold donor of the conference for their donation and support.

We appreciate the effort of all the authors who have contributed to these Proceedings. We would also like to express our gratitude to the members of the scientific and organizing committees, reviewers, speakers, chairpersons and all the conference participants for their support to the EcoTER'23. Sincere thanks go to all the people who have contributed to the successful organization of the EcoTER'23.

Prof. Snežana Šerbula,

President of the scientific and organizing committee

TABLE OF CONTENTS

Plenary Lecture

- Lidija Mančić, M. E. Rabanal, B. Marinković*
 OPTICALLY ACTIVE NANOMATERIALS FOR ENVIRONMENTAL
 REMEDIATION 2

Invited Lectures

- Aleksandra A. Jovanović*
 THE EXTRACTION OF ACTIVE COMPOUNDS FROM PLANT WASTE:
 THE POTENTIAL IN HUMAN AND INDUSTRIAL APPLICATIONS AS
 THE CONCEPT OF ZERO WASTE IN THE CIRCULAR ECONOMY 7
- Tanja Brdarić*
 ELECTROCHEMICAL ADVANCED OXIDATION PROCESSES FOR
 WASTEWATER TREATMENT: RECENT ADVANCES AND
 PERSPECTIVES 18
- Mirjana Marković, S. Radmanović, Đ. Čokeša, N. Potkonjak*
 HUMIC ACIDS IN THE ENVIRONMENT 30
- Mira Stanković, M. Prokopijević, D. Bartolić, J. Stevanović, F. Andrić, K. Radotić*
 ADVANCED OPTICAL TOOLS APPLIED ON HONEY SAMPLES FOR
 BEE HEALTH STATUS MONITORING 40
- Dragana Bartolić, M. Nikolić, M. Stanković, M. Prokopijević, M. Algara,
 S. Stanković, K. Radotić*
 ESTIMATION OF THE ANTIFUNGAL ACTIVITY OF THE TWO
 DIFFERENT CARBON DOTS AGAINST *Aspergillus flavus* 47

Conference Papers

Environmental monitoring and impact assessment

- Ana Čučulović, J. Stanojković, R. Čučulović, M. Stanković*
 RADIOACTIVITY IN SOIL AND MOSSES FROM THE SPECIAL
 NATURE RESERVE OF ZASAVICA IN 2021 56
- Djurdja Petrov, M. Ocokoljić, N. Galečić, D. Skočajić, I. Simović*
Chaenomeles × *superba* 'PINK LADY' IN DESIGNING PRIVATE
 GARDENS IN CONDITIONS OF CLIMATE CHANGE 62

| | |
|--|-----|
| <i>Mirjana Đurašević, I. Čeliković, I. Kandić, T. Milanović, A. Samolov, N. Mladenović Nikolić, A. Kandić</i> | |
| ACTIVITY CONCENTRATIONS OF ^{210}Pb , ^{137}Cs , AND ^{40}K IN WILD MUSHROOMS FROM SERBIA AND THEIR EFFECTIVE DOSE TO INGESTION | 69 |
| <i>Jelena Čović, M. Z. Momčilović, M. Randelović</i> | |
| LANTHANUM IMMOBILIZED ONTO GRAPHENE AS A CATALYST DESIGNED FOR ELECTROCHEMICAL APPLICATIONS | 75 |
| <i>Jelena Čović, M. Z. Momčilović, M. Randelović</i> | |
| NITROGEN DOPED CARBON MICROSPHERES SUPPORTED ONTO MWCNT AS NOVEL ELECTRODE MATERIAL | 82 |
| <i>Aleksandra Nesic, S. Meseldzija, M. Momcilovic</i> | |
| SUSTAINABLE PECTIN MONOLITH CRYOGELS | 88 |
| <i>Daniela Djikanović, O. Prodanović, J. Dragišić Maksimović, J. Jovanović, A. Kalauzi, D. Spasojević, K. Radotić</i> | |
| INVESTIGATION OF SILICA-LIGNIN INTERACTION. APPLICATION OF AFM AND FLUORESCENCE TECHNIQUES | 94 |
| <i>Vesna Djikanović, J. Čanak Atlagić, K. Zorić, S. Andjus, M. Ilić, V. Nikolić, K. Jovičić</i> | |
| COMPOSITION OF THE FISH COMMUNITY OF THE RIBNICA RIVER WITH RESPECT TO THE CONSERVATION STATUS | 99 |
| <i>Nikola Marinković, B. Tubić, A. Atanacković, N. Popović, J. Tomović, M. Raković, M. Paunović</i> | |
| INDICATIVE ECOLOGICAL STATUS ASSESSMENT OF RIBNICA RIVER (KOLUBARA BASIN) BASED ON AQUATIC MACROINVERTEBRATES | 104 |
| <i>Tamara Petronijević, I. Kostić Kokić, T. Anđelković, B. Zlatković, K. Kitanović, D. Bogdanović, N. Stanković</i> | |
| INFLUENCE OF FREEZING ON NITRATE AND NITRITE CONTENT IN RADISH, PARSLEY LEAF AND CELERY ROOT | 109 |
| <i>Marija Matić, D. Pavlović, V. Perović, D. Sekulić, N. Radulović, M. Mitrović, P. Pavlović</i> | |
| DETERMINATION OF PTEs CONTENT IN LIVESTOCK FODDER AND SOIL IN THE VICINITY OF THERMAL POWER PLANTS AND ASH DISPOSAL SITES | 115 |
| <i>Sonja Veljović Jovanović, S. Milić Komić, B. Živanović, A. Sedlarević Zorić, N. Šušić</i> | |
| LEAF NITROGEN BALANCE INDEX USED TO MONITOR STRESS RESPONSE TO AIR POLLUTION OF DECIDUOUS TREE SPECIES GROWN IN URBAN ZONE OF BELGRADE | 122 |

| | |
|--|-----|
| Bojana Živanović, S. Milić Komić, A. Sedlarević Zorić, A. Jelušić, N. Šušić, S. Marković, S. Veljović Jovanović | |
| USE OF BIOCHEMICAL METHODS FOR ASSESING OXIDATIVE STRESS IN TREES IN URBAN AREA DURING GROWING SEASON | 129 |
| Nikola Šušić, S. Milić Komić, B. Živanović, A. Jelušić, S. Marković, A. Sedlarević Zorić, S. Veljović Jovanović | |
| ACCLIMATION OF PEDUNCULATE OAK SEEDLINGS TO DIFFERENT LIGHT CONDITIONS IN THE FIRST MONTHS AFTER GERMINATION | 135 |
| Božica Vasiljević, J. Đuknić, N. Marinković | |
| BENTHIC DIATOMS AS PROXY FOR THE ECOLOGICAL CONDITIONS OF THE RIBNICA RIVER, SERBIA | 141 |
| Milanka Negovanović, L. Kričak, S. Milanović, J. Marković, N. Simić, S. Ignjatović | |
| BLASTING MATS FOR THE PROTECTION OF PEOPLE, STRUCTURES AND THE ENVIRONMENT IN PROXIMITY TO THE BLAST SITE | 147 |
| Aleksandra Kolarski, V. Srečković, Z. Mijić | |
| INFLUENCES OF EXTREME SOLAR ACTIVITY ON EARTH ENVIRONMENT – CASE STUDY | 154 |
| Maja Poznanović Spahić, A. Gulan, D. Spahić, P. Tančić, S. Sakan, S. Petrović | |
| AVAILABILITY OF TOXIC ELEMENTS IN ROADSIDE SOILS (HIGHWAY 75, VOJVODINA, SERBIA): IS THERE ANY SIGNIFICANT CONTAMINATION RISK? | 160 |
| Tanja Kalinović, A. Radojević, J. Kalinović, J. Milosavljević, S. Šerbula | |
| MULTICRITERIA EFFICIENCY ASSESSMENT OF THE PINE TREE POTENTIAL FOR THE PHYTOREMEDIATION OF COPPER | 167 |
| Žaklina Tasić, M. Petrović Mihajlović, A. Simonović, M. Radovanović, M. Antonijević | |
| ELECTROCHEMICAL SENSING OF FOLIC ACID | 173 |
| Vanja Trifunović, S. Milić, Lj. Avramović, M. Antonijević, M. Radovanović | |
| POTENTIAL ENVIRONMENT POLLUTANT – INTERMEDIATE PRODUCT OF THE STEEL PRODUCTION PROCESS | 179 |
| Natalija Ognjanović, V. Nedelkovski, S. Stanković, S. Milić | |
| BIOPESTICIDES IN THE ENVIRONMENT | 185 |
| Urban and industrial ecology | |
| Goran Milentijević, M. Agatonović, M. Rančić, M. Milosavljević | |
| ENVIRONMENTALLY ACCEPTABLE PROCEDURE FOR THE SYNTHESIS OF TETRAETHYLTHIURAMMONOSULFIDE TETS | 191 |

| | | |
|---|--|-----|
| <i>Andela Stojić, D. Tanikić, E. Požega</i> | | |
| TECHNOLOGICAL PROCESSES AS SOURCES OF POLLUTION IN THE ENVIRONMENT | | 198 |
| <i>Aleksandar Lisica, N. Stojanović, M. Veselinović, J. Petrović, N. Stavretović, M. Tešić</i> | | |
| LONDON PLANE (<i>Platanus × acerifolia</i> (Aiton) Willd.) IN THE STREET TREE LINES OF THE OLD TOWN IN BELGRADE | | 203 |
| <i>Djordja Petrov, M. Ocokoljić, N. Galečić, D. Skočajić</i> | | |
| APPLICATION OF SPECIES OF THE GENUS <i>Parthenocissus</i> L. IN URBAN GREEN INFRASTRUCTURE – STATE AND PERSPECTIVES | | 210 |
| <i>Djordja Petrov, M. Ocokoljić, N. Galečić, D. Skočajić, I. Simović</i> | | |
| SECOND FLOWERING OF <i>Philadelphus coronarius</i> L. IN GREEN-BLUE INFRASTRUCTURE OF BELGRADE | | 216 |
| <i>Dragana Pavlović, M. Matić, V. Perović, O. Kostić, D. Sekulić, M. Mitrović, P. Pavlović</i> | | |
| EFFECTS OF SO ₂ AND NO ₂ ON THE PHOTOSYNTHETIC EFFICIENCY AND CATALASE ANTIOXIDATIVE ENZYME ACTIVITY IN <i>Betula pendula</i> Roth | | 222 |
| <i>Ermenegilda Vitale, P. Napoletano, C. Arena, A. De Marco</i> | | |
| PLANT-SOIL RELATIONSHIPS IN MEDITERRANEAN SPECIES GROWN ON TECHNOSOLS ENRICHED WITH COMPOST | | 228 |
| Air, water and soil pollution, prevention and control | | |
| <i>Milica Blažić, M. Milovanović, T. Sekulić, V. Stupar, Z. Živković</i> | | |
| IMPACTS OF PESTICIDE APPLICATION ON THE ENVIRONMENT | | 235 |
| <i>George Vuković, D. Kovačević, N. Đorđević, M. Perić, S. Knežević, M. Nikolić, B. Vlahović, V. P. Pavlović, G. Rašić, S. Nenadović, M. Ivanović, M. Mirković, V. B. Pavlović</i> | | |
| GREEN SYNTHESIS OF GEOPOLYMER-POLYURETHANE COMPOSITES FOR EM SHIELDING | | 241 |
| <i>Ana Vukmirović, B. Obrovski, S. Vukmirović, I. Mihajlović</i> | | |
| APPLICATION OF STATISTICAL METHODS FOR THE ANALYSIS OF WASTEWATER TREATMENT PLANT EFFICIENCY | | 247 |
| <i>Ivana Mihajlović, A. Hgeig, N. Živančev, M. Petrović, M. Novaković</i> | | |
| COMPARISON OF DIFFERENT SORBENTS IN THE HERBICIDE REMOVAL FROM WATER | | 251 |
| <i>Aleksandar Krstić, I. Bracanović, D. Vasić Anićijević, A. Kalijadis</i> | | |
| VALLME PREPARATION METHOD FOR THE DETERMINATION PHARMACEUTICALS IN WATER | | 256 |

| | |
|---|-----|
| Marija Koprivica, J. Petrović, J. Dimitrijević, M. Ercegović, M. Simić, M. Grubišić | |
| REMOVAL EFFICIENCY OF HEAVY METAL IONS FROM AQUEOUS SOLUTION WITH WASTE TREE BIOMASS HYDROCHARS | 261 |
| Nevena Surudžić, D. Spasojević, M. Stanković, M. Spasojević, R. G. A. Elgahwash, R. Prodanović, O. Prodanović | |
| HORSERADISH PEROXIDASE IMMOBILIZATION WITHIN MICRO-BEADS OF OXIDIZED TYRAMINE-ALGINATE FOR PHENOL REMOVAL FROM WASTEWATER | 267 |
| Dragica Spasojević, O. Prodanović, N. Surudžić, D. Djikanović, J. Simonović Radosavljević, K. Radotić, R. Prodanović | |
| WASTEWATER TREATMENT BY AMINATED PEROXIDASE IN ALGINATE HYDROGEL | 272 |
| Branislava Matić, M. Milić | |
| CONTRIBUTION OF INSTITUTE OF PUBLIC HEALTH OF SERBIA IN MONITORING TRAFFIC-INDUCED AIR POLLUTION IN BELGRADE | 276 |
| Nenad Malić, U. Matko, M. Trbić, R. Pijunović, M. Marković | |
| ALTERNATIVE METHODS OF REHABILITATION (SOIL RECOVERY), RECLAMATION AND REMEDIATION OF MINE TECHNOSOLS | 283 |
| Snežana B. Simić, K. A. Markeljić | |
| PRELIMINARY ECOLOGICAL STATUS ASSESSMENT OF THE GROŠNICA RIVER BASED ON PHYTOBENTHOS | 289 |
| Snežana B. Simić, N. B. Đorđević | |
| AN ASSESSMENT OF THE ECOLOGICAL POTENTIAL OF ŠUMARICE RESERVOIRS (CENTRAL SERBIA) BASED ON PHYTOPLANKTON | 295 |
| Miloš Prokopijević, M. Stanković, D. Bartolić, A. Lj. Mitrović, K. Radotić | |
| FLUORESCENCE CHARACTERISATION OF BISPHEENOL A IN VARIOUS SOLVENTS AND DRINKING WATER | 302 |
| Slobodan Ničković, L. Ilić, S. Petković, G. Pejanović, A. Huete, Z. Mijić | |
| NOVEL APPROACH IN AIRBORNE POLLEN DISPERSION MODELLING | 306 |
| Nena Velinov, S. Najdanović, M. Petrović, M. Radović Vučić, M. Kostić, J. Mitrović, A. Bojić | |
| THE APPLICATION OF SORBENT SYNTHESIZED USING ULTRASOUND FOR REMOVAL OF TEXTILE DYE | 312 |
| Milica Petrović, S. Najdanović, N. Velinov, S. Rančev, D. Radivojević, M. Radović Vučić, A. Bojić | |
| ATMOSPHERIC PRESSURE CORONA PLASMA DEGRADATION OF REACTIVE ORANGE 4 IN DEIONZED AND RIVER WATER | 318 |

| | |
|---|-----|
| Slobodan Najdanović, M. Petrović, N. Velinov, M. Kostić, J. Mitrović, D. Bojić, A. Bojić | |
| THE INFLUENCE OF TYPE OF SOLVENT ON THE ELECTROCHEMICALLY SYNTHESIZED SORBENTS BASED ON BASIC BISMUTH NITRATES | 324 |
| Milena Dimitrijević, S. Kovačević, U. Jovanović, M. Stanić, M. Opačić, I. Santrač, M. Tanović, V. Čurić, I. Spasojević | |
| APPLICATION OF MICROALGA <i>Chlorella sorokiniana</i> IN WASTEWATER BIOREMEDIATION – CASE OF LAKE ROBULE | 330 |
| Milan Gorgievski, M. Marković, N. Štrbac, V. Grekulović, M. Zdravković | |
| ADSORPTION ISOTHERMS FOR COPPER IONS BIOSORPTION ONTO ONION PEELS | 335 |
| Sonja Stanković, V. Nedelkovski, M. Radovanović, S. Milić | |
| MECHANISM AND KINETICS OF ELECTROCATALYTIC OXIDATION OF PHENOL | 341 |
| Jelena Milosavljević, S. Šerbula, A. Radojević, T. Kalinović, J. Kalinović | |
| ECOENZYMATIC STOICHIOMETRY AS AN EMERGING METHOD IN THE ASSESSMENT OF SOIL HEAVY METAL POLLUTION | 348 |
| Protection and preservation of natural resources | |
| Mihajlo Stanković | |
| ORCHIDS OF THE ZASAVICA SPECIAL NATURE RESERVE | 354 |
| Gordana Šekularac, M. Aksić, T. Dimitrijević (ex. Ratknić), M. Vranešević, N. Gudžić, M. Ratknić | |
| CLIMATIC BALANCE OF THE WATER FOR THE SOIL OF THE KRUŠEVAC REGION IN CENTRAL SERBIA | 361 |
| Gordana Šekularac, M. Aksić, T. Dimitrijević (ex. Ratknić), M. Vranešević, S. Gudžić, N. Gudžić, M. Ratknić | |
| INFLUENCE OF IRRIGATION METHOD ON THE OCCURRENCE AND INTENSITY OF THE GRAY MOLD OF LETTUCE | 367 |
| Aleksandar Stevanović, T. Sekulić, M. Blažić, N. Radić, A. Popović, V. Stupar | |
| THE IMPACT OF IRRIGATION ON THE QUALITY OF THE ENVIRONMENT AND WATER RESOURCES | 373 |
| Aleksandar Stevanović, M. Saulić, M. Blažić, V. Stupar, D. Stojićević, Z. Živković | |
| BIOPREPARATIONS IN THE FUNCTION OF ORGANIC AGRICULTURE IN FRUIT GROWING AND VITICULTURE | 379 |
| Vladanka Stupar, T. Sekulić, M. Blažić, N. Radić, A. Popović, A. Stevanović | |
| IRRIGATION – IMPACT ON SOIL AS AN ENVIRONMENTAL FACTOR | 385 |

Milan Nedeljković, S. Mladenović, J. Petrović

A RENEWABLE ENERGY SOURCES AND SUSTAINABLE DEVELOPMENT EQUATION

391

Ecological ethics and environmental education

Tatjana Miljojčić

FORGING A SUSTAINABLE FUTURE: THE CIRCULAR ECONOMY IN THE FASHION INDUSTRY

396

Ecotoxicology and environmental safety

Darko Anđelković, M. Branković

CITRATE BUFFERED QuEChERS vs SIMPLIFIED SAMPLE PREPARATION METHOD: COMPARATIVE LC/MS ANALYSIS OF PESTICIDES IN APPLES

402

Darko Anđelković, M. Branković

APPLICABILITY OF THE QuEChERS IN NON-CHROMATOGRAPHY-BASED PESTICIDE ANALYSIS IN APPLES

407

Darko Anđelković, M. Branković

ESI vs APCI IN SELECTED PESTICIDES MS DETECTION IN APPLES

413

Tamara Petronijević, I. Kostić Kokić, Dj. Milošević, M. Stojković Piperac, N. Stanković, T. Anđelković

DIFFERENT GROWTH RESPONSES OF SELECTED REPRESENTATIVES OF PHYTOPLANKTON TO THE PRESENCE OF THE ANTIBIOTIC VANCOMYCIN

420

Tamara Petronijević, I. Kostić Kokić, T. Anđelković, B. Zlatković, D. Stajić, D. Bogdanović, N. Stanković

DETERMINATION OF SEVEN ANIONS IN WATER LETTUCE GROWN IN A NATURAL UNPOLLUTED HABITAT BY ION CHROMATOGRAPHY

426

Milica Zdravković, V. Grekulović, N. Štrbac, J. Suljagić, I. Marković, M. Gorgievski, M. Marković

THE COPPER CORROSION IN CHLORIDE MEDIUM WITH ADDITION OF BLACKBERRY LEAF EXTRACT

432

Hazardous materials and green technologies

Aleksandra A. Jovanović, M. R. Elferjane, M. Gnjatović, B. Bugarski, A. Marinković

PHOSPHOLIPID LIPOSOMES AS A CARRIER FOR ALOE VERA WASTE EXTRACT

438

| | |
|--|-----|
| Aleksandra A. Jovanović, M. R. Elferjane, M. Milošević, M. Gnjatović, A. Marinković Vaccinium myrtillus LEAF WASTE EXTRACTS WITH NATURAL DEEP EUTECTIC SOLVENT | 444 |
| Danijela Kovačević, N. Đorđević, S. Glišić, B. Vlahović, V. B. Pavlović MORPHOLOGICAL INVESTIGATION OF PVDF/MAGNETITE@NC/BaTiO ₃ SEMI-SPHERICAL COMPOSITE MATERIALS FOR OIL REMOVAL | 450 |
| Branislava Savić, D. Aćimović, M. Ječmenica Dučić, M. Simić, D. Vasić Anićijević, T. Brdarić DEGRADATION OF PHENOL AND SUBSTITUTED PHENOLS: INFLUENCE OF APPLIED POTENTIAL | 456 |
| Marija Ječmenica Dučić, D. Aćimović, B. Savić, M. Simić, A. Krstić, D. Vasić Anićijević, T. Brdarić DEGRADATION OF DYES MIXTURE BY ELECTROCHEMICAL OXIDATION USING STAINLESS STEEL ELECTRODE | 460 |
| Marija Simić, D. Aćimović, B. Savić, M. Ječmenica Dučić, I. Perović, D. Vasić Anićijević, T. Brdarić THE OXYGEN EVOLUTION REACTION AT TIN DIOXIDE-CARBON-BASED ELECTRODES | 465 |
| Drita Abazi Bajrami, M. Marinkovski, K. Lisichkov, S. Kuvendziev OPTIMIZATION OF THE <i>Helichrysum arenarium</i> EXTRACT OBTAINED WITH ULTRASOUND-ASSISTED EXTRACTION | 469 |
| Berina Sejdinović VIBRATION ISOLATION | 475 |
| Uroš Stamenković, I. Marković THE INFLUENCE OF AGEING ON THE THERMAL PROPERTIES AND MICROSTRUCTURE OF THE EN AW-6082 GREEN ALUMINIUM ALLOY | 482 |
| Ljubiša Balanović, D. Manasijević, I. Marković, U. Stamenković, M. Petrić MICROSTRUCTURAL AND THERMAL CHARACTERIZATION OF Bi-Sb-Sn ALLOYS FOR ECOLOGICAL APPLICATION | 488 |
| Vladan Nedelkovski, S. Stanković, M. Radovanović, Ž. Tasić, S. Milić OPTIMIZATION OF PHENOL ELECTROCHEMICAL OXIDATION USING MODIFIED Ti/SnO ₂ -TYPE ANODES | 494 |
| Aleksandar Cvetković, Ž. Tasić, M. Petrović Mihajlović, A. Simonović, M. Radovanović, M. Nujkić, M. Antonijević INFLUENCE OF SUBSTITUTES ON THE EFFICIENCY OF ORGANIC CORROSION INHIBITORS | 500 |

| | |
|---|-----|
| Sonja Stanković, M. Nujkić, Ž. Tasić, D. Medić, A. Papludis, S. Milić | |
| MODIFIED MEMBRANES WITH GRAPHENE OXIDE – REMOVAL OF DYES FROM WASTEWATER | 506 |
| Human and ecological risk assessment | |
| Olga Kostić, D. Pavlović, M. Marković, Z. Miletić, N. Radulović, M. Mitrović, P. Pavlović | |
| HUMAN HEALTH RISK ASSESSMENT OF PTE _s IN ELECTROFILTER ASH AND CHRONOSEQUENCE FLY ASH FROM “TENT A” DISPOSAL SITES | 512 |
| Agriculture: nutrition, organic food and health impacts | |
| Markola Saulić, V. Trajić, D. Stojićević, A. Stevanović, Z. Živković | |
| EFFECT OF EXTRACT <i>Ecklonia maxima</i> ON CONDITION OF AGRICULTURAL CROPS | 519 |
| Metodi Mladenov | |
| SUITABILITY OF THE SOILS IN THE MUNICIPALITY OF KOVACHEVTSI, BULGARIA FOR GROWING ON EINKORN WHEAT (<i>Triticum monococcum</i>) | 524 |
| Gorica Cvijanović, V. Stepić, M. Bajagić, V. Cvijanović, J. Marinković, N. Đurić | |
| INFLUENCE OF EFFECTIVE MICROORGANISMS ON THE BASIC PARAMETERS OF SOIL BIOGENICITY IN THE PRODUCTION OF WHEAT AND CORN | 529 |
| Vojkan Miljković, R. Ljupković, M. Miljković | |
| APPLICATION OF CLASSIC THIN LAYER CHROMATOGRAPHY METHOD FOR QUALITATIVE DETERMINATION OF SYNTHETIC FOOD COLORS | 535 |
| Alternative energy: efficiency and environmental policy | |
| Snežana Brković, N. Zdolšek, I. Perović, G. Tasić, M. Seović, S. Mitrović, J. Georgijević | |
| NOVEL CARBON MATERIAL FOR OER IN VARIOUS ELECTROLYTE SOLUTIONS | 540 |
| Nikola Zdolšek, I. Perović, S. Brković, M. Seović, J. Georgijević, S. Mitrović, P. Laušević | |
| THE EFFECT OF DIFFERENT TYPE OF ELECTROLYTES ON THE DISCHARGE CAPACITY OF Zn-AIR BATTERIES | 545 |
| Jelena Georgijević, J. Milikić, N. Zdolšek, I. Perović, S. Brković, S. Mitrović, B. Šljukić | |
| IRON, COBALT DUAL DOPED CARBON ELECTROCATALYST FOR EFFICIENT WATER SPLITTING | 550 |

Greenhouse effect and global climate change

- Tatjana Dimitrijević, G. Šekularac, M. Ratknić, M. Aksić**
EFFECTS OF CLIMATE CHARACTERISTICS ON THE DIAMETER INCREMENT OF RED OAK IN THE CITY OF BELGRADE (SERBIA) 555
- Milica Blažić, T. Sekulić, V. Stupar, Z. Živković**
GREENHOUSE EFFECT AND GLOBAL CLIMATE CHANGE – IMPACT ON AGRICULTURE 561
- Vojkan Miljković, I. Gajić, Lj. Nikolić**
GLOBAL CLIMATE CHANGES: GREENHOUSE GASSES, CITIES AND PLASTICS 567

Sustainable development and green economy

- Zlata Živković, M. Saulić, D. Stojićević, M. Jevtić, V. Stupar**
ROLE OF NUTRIENTS IN CONTROLLING PLANT DISEASES AND PATHOPHYSIOLOGICAL ALTERATIONS IN PLANTS IN SUSTAINABLE AGRICULTURE. A REVIEW 572
- Zlata Živković, M. Saulić, D. Stojićević, M. Jevtić**
THE WAY OF MANAGING PLANT DISEASES IN SUSTAINABLE AGRICULTURE 578
- Dragan Ugrinov, M. Nikolić**
THE ROLE OF PLANTS IN BIOECONOMY AND CIRCULAR ECONOMY 584
- Vojkan Miljković, I. Gajić, Lj. Nikolić**
AGRICULTURAL WASTE IN SUSTAINABLE AGRICULTURE 589
- Ana Radojević, J. Milosavljević, S. Šerbula, T. Kalinović, J. Kalinović**
RECYCLING OF Li-ION BATTERIES FROM THE END-OF-LIFE VEHICLES: OPPORTUNITY OR LIABILITY IN THE FUTURE? 593

Environmental biology

- Vladimir Topalović, S. Matijašević, V. Savić, J. Nikolić, J. Stojanović, S. Zildžović, S. Grujić**
CRYSTALLIZATION CHARACTERISTICS OF BIOACTIVE POLYPHOSPHATE GLASSES 599

Environmental and material flow management

- Isidora Berežni, T. Marinković, B. Batinić**
ASSESSING THE COMPOSITION OF MUNICIPAL SOLID WASTE IN ŠID 605

Ivan Bracanović, A. Krstić, A. Kalijadis

SYNTHESIS AND CHARACTERISATION OF CARBON NANOMATERIAL USING HYDROTHERMAL CARBONISATION METHOD

612

Hamid Husić, S. Čerčić, V. Aganović

RETROSPECTIVE OF THE PLANNED ACTIVITIES FOR THE REHABILITATION OF THE DAMAGED AREA OF THE FORMER SURFACE MINE ČUBRIĆ

617

Student Section

Students: Ana Smiljković, Isidora Sujić (Serbia)

Mentor: Maja Nujkić (Serbia)

ENVIRONMENTAL AND HEALTH RISK OF CO₂ IN INDOOR ENVIRONMENTS

624

Student: Avram Kovačević (Serbia)

Mentor: Uroš Stamenković (Serbia)

ANTHROPOGENIC MERCURY IN THE ENVIRONMENT: GLOBAL EMISSIONS AND RECYCLING POSSIBILITIES

626

Student: Petar Milanović (Serbia)

Mentors: Uroš Stamenković, Avram Kovačević (Serbia)

THE INFLUENCE OF COOLING RATE ON MECHANICAL PROPERTIES AND MICROSTRUCTURE OF C45 CARBON STEEL

628

Student: Milica Denić (Serbia)

Mentor: Jelena Kalinović (Serbia)

AIR POLLUTION WITH CARCINOGENIC SUBSTANCES

630

Student: Gordan Mišić (Serbia)

Mentor: Jelena Kalinović (Serbia)

ACID RAIN AND SMOG – CHEMICAL REACTIONS

632

Student: Milica Denić (Serbia)

Mentor: Ana Radojević (Serbia)

MEDICAL WASTE MANAGEMENT

634

Student: Gordan Mišić (Serbia)

Mentor: Ana Radojević (Serbia)

ENVIRONMENTAL POLLUTION BY PET PACKAGING

636

Student: Marija Stanković (Serbia)

Mentor: Ana Simonović (Serbia)

COPPER CORROSION IN ARTIFICIAL ACID RAIN SOLUTION IN PRESENCE OF 5-PHENYL-1-TETRAZOLE

638

ELECTROCHEMICAL SENSING OF FOLIC ACID

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Abstract

The purpose of this article is to analyze the findings from studies on the electrochemical determination of folic acid (Vitamin B9). Folic acid is an important water-soluble vitamin for various biological processes, and its deficiency in human organisms can lead to health problems. Different electrochemical sensors are available for detecting folic acid in various mediums. These sensors provide rapid, selective and sensitive detection of folic acid, making them a valuable tool in analytical research. The results of researchers who used low-cost and environmentally friendly materials to prepare sensors are presented in this study. The limit of detection of one such sensor was 1.8×10^{-10} mol dm⁻³ indicating its good selectivity and sensitivity.

Keywords: electrochemical sensors, folic acid, vitamins, differential pulse voltammetry.

INTRODUCTION

Vitamins represent essential nutrients that are necessary for normal functioning of the human organism. They are introduced into the body through food or supplements because the body cannot synthesize them [1]. The B group vitamins include eight water-soluble vitamins that play important roles in cell metabolism. They are: B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folate), and B12 (cobalamin). Each B vitamin has its own unique function, but they all work together to help the body convert food into energy and to support the nervous system. Deficiencies in B vitamins can lead to various health problems, such as anemia, neuropathy, and birth defects [2]. Folic acid (vitamin B9) is important for several processes in the human body, including the formation of red blood cells, for cardiovascular health, nucleic acid synthesis, the normal development of the neural tube and to produce neurotransmitters [3]. B vitamins are commonly found in animal products such as meat, fish, and dairy products, as well as in supplements. Spinach, white beans, asparagus, soybeans, oranges and melons are also the important sources of folic acid [2].

There are several analytical methods that can be used to determine the level of folic acid (FA) in a sample, including: high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), spectrophotometry [4]. Despite their high precision and sensitivity, these methods for determining folic acid have drawbacks such as time-consuming procedures, necessary sample preparation, expensive equipment, and complexity of procedures [5]. On the other hand, electroanalytical methods, which are frequently utilized due to their benefits of low cost, simple sample preparation, fast analysis time, high accuracy, and low detection

limits, are increasingly being used as alternatives. Electrochemical sensors can be used to detect folic acid, too. There are several advantages of using electrochemical sensors for the detection of folic acid compared to other analytical methods: sensitivity, selectivity, they are also portable and cost-effective.

Different electrodes were used for the determination of folic acid including glassy carbon electrode, carbon paste, pencil graphite electrode, silver solid amalgam, and mercury drop electrode [5,6]. The significance of the determination of folic acid in different mediums and using different sensors is highlighted in review works by Di Tino *et al.* [4] and Batra *et al.* [7]. Additionally, in the paper of Alizadeh *et al.* [6] the possibilities of using modified carbon-based electrodes for the determination of FA are summarized.

The aim of this paper is to point out the importance of using low-cost materials for developing electrochemical sensors for the determination of folic acid in different matrices.

ELECTROCHEMICAL SENSORS FOR THE DETERMINATION OF FOLIC ACID

In the early 2000s, bismuth-film electrodes (BiFEs) attracted an increasing interest from researchers in electroanalytical investigations, as an adequate replacement for mercury electrodes. Compared to the mercury electrodes, bismuth-based electrodes are non-toxic and have a wide potential window. Bismuth-film electrodes are a type of electrochemical sensor that can be used for the detection of various compounds, including folic acid. They are made by depositing a thin film of bismuth onto a conductive substrate, such as glassy carbon electrodes (GCEs), carbon paste electrodes (CPEs), screen-printed electrodes (SPEs) [8]. Bismuth-film electrodes can be used to detect a wide range of substances, including: heavy metals, and organic compounds (pesticides, vitamins, amino acids).

Pencil graphite electrodes belong to the carbon based electrodes. Carbon based electrodes stand out from other materials due to their properties, such as chemical stability, a large active surface, the ability to modify the surface, and electrocatalytic activity in various oxidation-reduction reactions [9]. In their papers, Tasić *et al.* [10,11] showed the applicability of low-cost electrochemical sensors, such as PGE and an electrochemical sensor prepared using recycled carbon from spent batteries, for the detection of target analytes.

The effectiveness of poly(cystine)/PGE in determining folic acid was studied through the use of differential pulse voltammetry (DPV) and cyclic voltammetry (CV) techniques. The peak current of the unmodified pencil graphite electrode was found to be 0.77 μA . However, the response of folic acid was enhanced to 3.56 μA with the use of poly(cystine)/PGE. This improvement was attributed to the presence of thiol groups in L-cystine, which facilitate electron transfer and enhance the conductive properties of the electrode [5]. The slope of the E_p versus pH plot was calculated to be -35 mV/pH, indicating that the oxidation of folic acid involves a transfer of two electrons and one proton [5].

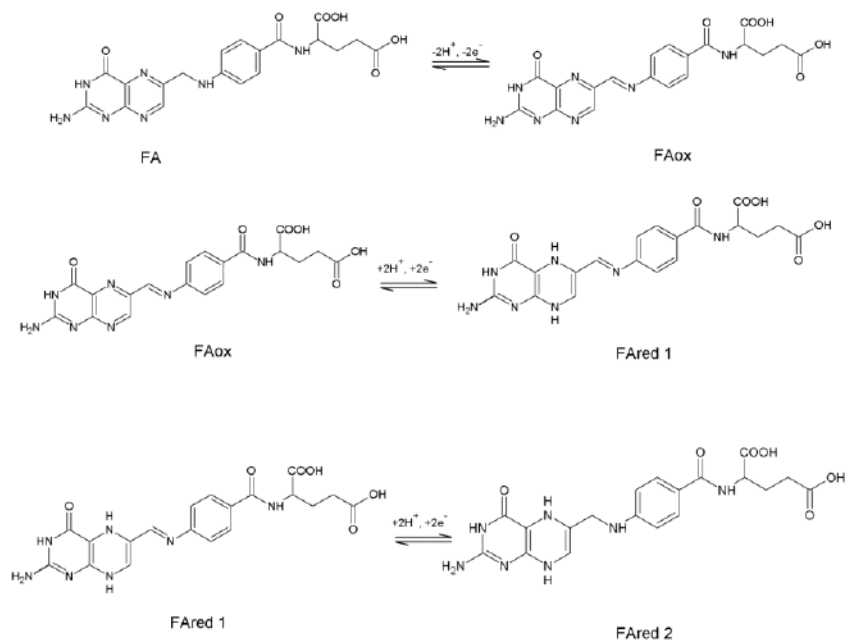


Figure 1 Proposed mechanism of oxidation and reduction of FA on graphite paste electrode [12]

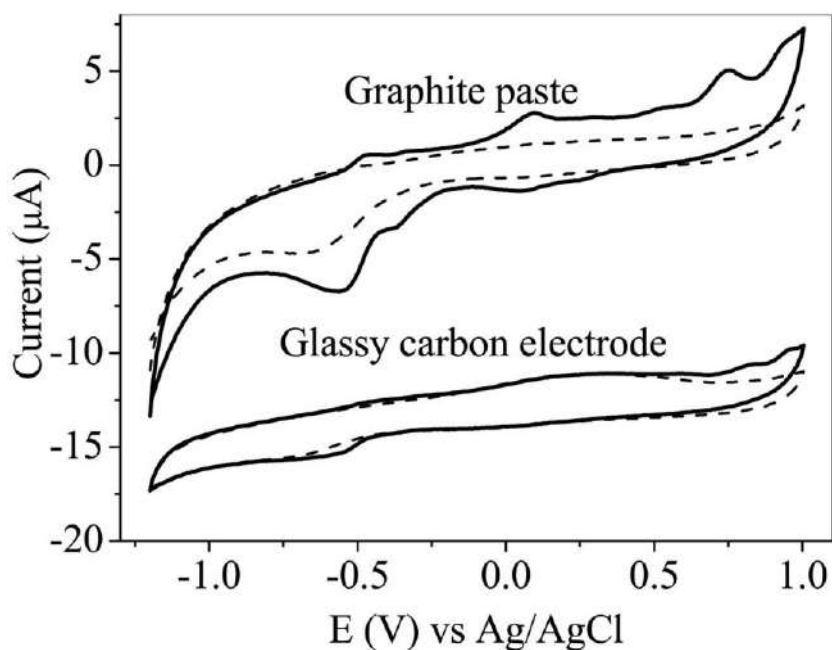


Figure 2 Cyclic voltammograms obtained for 44.14 mg L^{-1} FA in 0.1 mol L^{-1} acetate buffer as supporting electrolyte at pH 4.5 with $v = 50 \text{ mV s}^{-1}$, using graphite paste-based electrode (dashed line – blank solution) and glassy carbon electrode (dashed line – blank solution) [12; <https://doi.org/10.1016/j.jelechem.2018.04.043>].

Table 1 summarizes the results obtained by different electrochemical sensors for determining FA.

Table 1 Electrochemical characteristics of different electrodes as sensor for folic acid detection

| Sensor | Medium | Method | Linear range | LOD | Reference |
|---|--------------------------------|-----------|---|---|-----------|
| FA-imp/CNDs/PGE | PBS (pH 6.2) + 0.1 M KCl | CV, DPV | 2.2–30.8 ng mL ⁻¹ | 2.02 ng mL ⁻¹ | [3] |
| poly(cysteine)/PGE | PBS pH 7 | CV, DPV | 1.0–100 μmol dm ⁻³ | 0.43 mol dm ⁻³ | [5] |
| NiZCB-GCE | | DPV | 0.004–0.22 mg dm ⁻³ | 1.3 μg dm ⁻³ | [13] |
| Bismuth film electrode | | SWAdS | 0.5–20 nmol dm ⁻³ | 0.2 nmol dm ⁻³ | [14] |
| GPE modified with hybrid MIP (poly(methacrylic acid)-SiO ₂) | pH 4.5 | DPV, SWV | 0.005–0.1 μg dm ⁻³ | 0.72 μg dm ⁻³ | [12] |
| poly tyrosine modified PGE | PBS pH 7 | CV, DPV | 1 μM–85 μmol dm ⁻³ | / | [15] |
| BiFE-GCE | BR pH 2 | CV, DCV | 2.5x10 ⁻⁸ –3x10 ⁻⁵ mol dm ⁻³ | 4.1x10 ⁻⁹ mol dm ⁻³ | [8] |
| BiFE-GCE | Acetate buffer solution pH 4.5 | SWCSV, CV | 0.1–10.0 μmol dm ⁻³ | 1x10 ⁻³ μmol dm ⁻³ | [16] |

CV – cyclic voltammetry; DPV – differential pulse voltammetry; SWV – square wave voltammetry; DCV – direct current voltammetry; SWCSV – square wave cathodic stripping voltammetry; PGE – pencil graphite electrode; NiZCB-GCE – Ni-zeolite/carbon black-modified glassy carbon electrode; BiFE-GCE – a bismuth-film electrode prepared on the glassy carbon electrode; FA-imp/CNDs/PGE – folic acid imprinted on a pencil graphite electrode modified with carbon nanodots; PBS – phosphate buffer solution; BR – Britton-Robinson buffer solution; LOD – limit of detection.

Porada *et al.* [13] in their study presented a low-cost and highly specific voltammetric method for measuring vitamins B2, B9, B12, and B3 using a Ni-zeolite/carbon black-modified glassy carbon electrode (NiZCB/GCE). By using an environmentally friendly Ni-exchanged natural zeolite and conductive carbon black (CB) in a one-step modification of the GCE, a highly sensitive sensor capable of accurately detecting individual B-group vitamins, even in the presence of other vitamins, was obtained with a low limit of detection (Table 1). Kuceki *et al.* [12] initially compared the electrochemical responses of folic acid using graphite paste and glassy carbon electrodes (Figure 2). The results showed substantial improvements in both the anodic and cathodic peak currents when using the graphite paste electrode. This improvement may be due to the higher porosity of the graphite paste compared to the glassy carbon electrode, which makes the adsorption process easier [12]. As reported, the oxidation process at +0.8 V occurs on the side-chain, as demonstrated in Figure 1, which results in the loss of two protons and two electrons [12]. The reduction processes at -0.35 V and -0.50 V are likely attributed to the reduction of the pterin moiety of the FA molecule (Figure 1), and the reduction of the side-chain –CH=N–, respectively. According to the data reported by Kuceki *et al.* [12], the inclusion of a low amount (3% w/w)

of molecularly imprinted polymer in the graphite paste electrode enabled the development of a highly sensitive and selective sensor for folic acid without the need for a preconcentration step. Stepankova *et al.* [8] and Vladislavljevic *et al.* [16] used a bismuth-film prepared ex situ on a glassy carbon electrode for the determination of folic acid. No oxidation signal was recorded in Britton–Robinson buffer (pH 5) under the optimised conditions, indicating that the electrode process is irreversible with one reduction peak near -0.55 V [8,16]. The shift in the reduction potential to a less negative value and increasing the intensity of folic acid reduction peak on the BiFE compared to the GCE suggest the electrocatalytic nature of the BiFE. This behaviour was also seen with the BiNWs/GCE and is believed to be due to its high surface-to-volume ratio and uniform pore size that enhance the rapid reduction kinetics of folic acid [16]. Vladislavljević *et al.* [17] prepared BiFE on GCE at different pH, with or without the presence of a complexing agent (EDTA) and used the sensor to determine folic acid and glutathione. The findings in this study show a strong correlation between the bismuth film structure and the electrolyte composition and electrodeposition parameters. Typically, a thick, uniform, non-porous bismuth film was obtained when electrodeposition was carried out in an acetic buffer with a pH of 4.5. However, incorporating the complexing agent ethylenediaminetetraacetic acid (EDTA) into the acetic buffer produced a flake-like, dendritic structure. On the other hand, films produced in HNO₃ had a porous structure and lacked stability. The use of the BiF electrode in the determination of folic acid showed that the film created in the acetate buffer with the addition of EDTA has impressive stripping performance and improved sensitivity. Geca *et al.* [18] demonstrated that the solid bismuth microelectrode is a highly efficient tool for determining folic acid, providing a wide linear range, low limits of detection and quantification, and high selectivity. The lowest reported limit of detection for folic acid was 1.8×10^{-10} mol dm⁻³. The adsorptive stripping voltammetry method with prepared sensor can be used in pharmaceutical analysis without complicated sample preparation. Furthermore, the solid bismuth microelectrode does not require the addition of Bi³⁺ to the supporting electrolyte, making it an environmentally-friendly alternative to bismuth film electrodes. Overall, the solid bismuth microelectrode is a convenient and superior alternative to previously reported bismuth microelectrode arrays and bismuth film electrodes [18]. According to the results summarized in Table 1, it can be said that the modified PGEs were better in near-neutral solution, while the sensory characteristics of BiFE were better in acidic environments.

CONCLUSION

The importance of folic acid in various vital biological processes, such as nucleic acid synthesis, cell division, fetus growth and development, has led to its widespread use as a supplement to prevent and treat folate deficiency in human organism. As a result, in recent years, there has been a significant effort in the analytical field to create precise and reliable sensors for the determination of folic acid due to its crucial role in human health. Based on a literature review, it can be seen that different prepared electrochemical sensors can be used to determine folic acid. Some of them were better in acidic solutions (BiFEs), while others were better in neutral solutions (modified PGEs). Depending on the experimental conditions and the type of sensor, the detection limits ranged from 1.8×10^{-10} mol dm⁻³ to 0.43 mol dm⁻³.

In addition, the researchers showed that the modified electrodes have improved sensing capabilities than the unmodified ones. It is still a challenge for researchers to develop a suitable sensor that is sensitive and selective, while being cheap and environmentally friendly.

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