UNIVERSITETI I PRISHTINËS
"HASAN PRISHTINA"
FAKULTETI I SHKENCAVE MATEMATIKE NATYRORE

Rr. Eqrem Çabej, 10000 Prishtinë, Republika e Kosovës
Tel: +381-38-249-873 • E-mail: fshmn@uni-pr.edu • www.uni-pr.edu

FSHMN **Ref. nr.** 422 **Prishtinë, Dt.** 13.02.2024

KËRKESË

Për: Këshillin Mësimor të Departamentit të Biologjisë

Këshillin e Studimeve të Doktoratës të FShMN-së

Këshillin e Fakultetit të Shkencave Matematike- Natyrore

Lënda: Kërkesë për formimin e komisionit për vlerësimin e doreshkrimit të temës së doktoratës

Sipas Statutit të Universitetit të Prishtinës "Hasan Prishtina" dhe Rregullores ekzistuese për studime të doktoratës, i plotësoj kushtet për vlerësimin e doreshkrimit, prandaj kërkoj nga organet e lartëpërmendura të FShMN-së të më mundësojnë formimin e komisionit për vlerësim të doreshkrimit të temës së doktoratës me titull: "**Vlerësimi i tolerancës ndaj stresit oksidativ te misri (*Zea mays* L.) të shkaktuar nga ndotja e tokës me metale të rënda në zonën përreth shkritores së Ferronikelit në Drenas"**

Kërkesës ia bashkangjes:

1. Kopjen e doreshkrimit
2. Një punim shkencor nga lëmia e ngushtë
3. Pëlqimin e mentorit për dorëzimin e doreshkrimit
4. Dëshmitë për pjesëmarrje në konferenca
5. Formularin F6

Prishtinë: 13.02.2024

Kandidati: Msc. Liridon Buqaj

Email.: liridon.buqaj@iuni-prizren.vom

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Këshillin e Fakultetit të Shkencave Matematike dhe Natyrore

Lënda: Pëlqim nga Mentori për dorëzimin e doréshkrimit të temës së doktoratës me titull: **"Vlerësimi i tolerancës ndaj stresit oksidativ te misri (*Zea mays* L.) të shkaktuar nga ndotja e tokës me metale të rënda në zonën përreth shkritores së Ferronikelit në Drenas"** për kandidatin Msc. Liridon Buqaj.

Mendim:

Doréshkrimi i temës së doktoratës i kandidatit Msc. Liridon Buqaj, është punim shkencor burimor me vlerë të posaçme shkencore dhe praktike. Rezultatet e fituara nga ky punim japin një kontribut të vlefshëm në aspektin e përcaktimit të përqendrimit të metaleve në tokë dhe translokimin e tyre në bimë, si dhe kontribut shkencor në njohjen e nivelit toksik që shkaktojnë te kjo bimë e kultivuar në këtë zonë. Bazuar në faktin se, bima e misrit është bioakumuluese dhe një ndër llojet kryesore të drithërave që kultivohet edhe tek ne, ky hulumtim do shërbejë si model për të vlerësuar toksicitetin e metaleve të rënda nëpërmjet përcjelljes së biomarkerëve specifik në bimë dhe jo vetëm. Prandaj rezultatet arritura, do të kontribuojnë drejtpërsëdrejti në njohjen e mekanizmave mbrojtës dhe ndërveprues të nivelit molekular në kuadër të qelizave bimore. Kontribut tjetër i rëndësishëm është se, rezultatet e këtij studimi janë të dobishme për të orientuar identifikimin e pikave të nxehta të akumulimit të metaleve të rënda në tokat bujqësore dhe ndikimet e drejtpërdrejta në cilësinë e tokës, por edhe mundësinë e bartjes në hallkat e tjera të zinxhirit ushqyes. Nga kjo do të përfitojnë drejtpërdrejtë edhe banorët lokal dhe institucionet përkatëse për të kuptuar gjendjen e saktë mjedisore të kësaj zone dhe hapat që duhet të ndërmerren në menaxhimin efektiv të këtyre tokave.

Për më tepër, ky projekt i doktoratës ka qenë pjesë e një projekti shkencor të aprovuar nga MASHTI për mbulimin e shpenzimeve të hulumtimit.

Nga këto rezultate të fituara janë publikuar dy punime shkencore të indeksuara në *Web of Science* dhe kandidati ka marrë pjesë në tri konferenca shkencore ndërkombëtare, të cilat janë paraqitur si më poshtë:

Pjesë të punimit të botuara në formë punimi shkencor:

1. Liridon Buqaj, Bekim Gashi, Muhamet Zogaj, Ramë Vataj, Valbona Sota & Metin Tuna (2023). Stress induced by soil contamination with heavy metals and their effects on some biomarkers and DNA damage in maize plants at the vicinity of Ferronikel smelter in Drenas, Kosovo. Journal of Environmental Science and Health, Part B, Taylor & Francis, 58:10, 617-627. https://doi.org/10.1080/03601234.2023.2253114
2. Bekim Gashi, Liridon Buqaj, Ramë Vataj & Metin Tuna (2024). Chlorophyll biosynthesis suppression, oxidative level and cell cycle arrest caused by Ni, Cr and Pb stress in maize exposed to treated soil from the Ferronikel smelter in Drenas, Kosovo. Plant Stress, Elsevier, 100379. https://doi.org/10.1016/j.stress.2024.100379

Pjesë të punimit të botuara në formë kumtese:

1. Liridon Buqaj, Bekim Gashi, Metin Tuna (2023). DNA content and cell cycle on maize plant (*Zea mays* L.) under heavy metals stress. 14th International Conference of the French Society of Plant Biology – Plant Biology Europe. July 3 – 6, 2023, Marseille, France. P 152-153.
2. Liridon Buqaj, Bekim Gashi, Muhamet Zogaj, Ramë Vataj, Makfire Sadiku (2023). Effect of different concentrations of Ni, Cr and Pb on some biochemical parameters and antioxidant response of maize (*Zea mays* L.). 8th International Congress on Applied Biological Sciences, 13-16 September, Prishtina, Kosovo. P 39.
3. Liridon Buqaj, Bekim Gashi, Muhamet Zogaj, Makfire Sadiku (2022). Antioxidant response of maize (*Zea mays* L.) due to soil contamination by heavy metals in the vicinity of the Ferronikel smelter in Drenas, Kosovo. International Scientific Conference "BRIDGE2022", from 09-10 December 2022, Prizren, Kosovo. P 44.

Bazuar në rëndësinë që ka ky hulumtim, punën dhe përkushtimin e kandidatit në fjalë, në cilësinë e mentorit të tij mendoi se ky hulumtim i plotëson kriteret e punimit të doktoratës. Prandaj, sipas Rregullores për studime të doktoratës në Universitetin e Prishtinës "Hasan Prishtina", ky punim i plotëson kriteret që të procedohet më tujte.

Prishtinë:

12/2/2024

Mentori:

Prof. Asoc. Dr. Bekim Gashi

PARAQITJA E PUNIMIT TË DOKTORATËS [1]

TË DHËNAT E PËRGJITHSHME

Doktoranti:	Ass. Msc. Liridon Buqaj
Adresa:	Rr. Shkronjat p.n, Universiteti Ukshin Hoti në Prizren
Tel./ fax:	+38344923026
E-mail:	liridon.buqaj@uni-prizren.com
Emërtimi i studimit:	Biologji e organizmave dhe Ekologji
Udhëheqësi i studimit:	Departamenti i Biologjisë, FSHMN, Universiteti i Prishtinës "Hasan Prishtina"

TË DHËNAT PËR PUNIMIN E DOKTORATËS

Titulli në gjuhën shqipe	"Vlerësimi i tolerancës ndaj stresit oksidativ te misri (*Zea mays* L.) të shkaktuar nga ndotja e tokës me metale të rënda në zonën përreth shkritores së Ferronikelit në Drenas"
Titulli në gjuhën angleze	"Evaluation of oxidative stress tolerance in maize (*Zea mays* L.) due to soil contamination by heavy metals in the vicinity of the Ferronikel smelter in Drenas"
Fusha e hulumtimit	Fiziologjia e stresit të bimëve

DEKLARATA E MENTORIT/BASHKËMENTORIT

Dorëshkrimi i temës së doktoratës i kandidatit Msc. Liridon Buqaj, është punim shkencor burimor me vlerë të posaçme shkencore dhe praktike. Rezultatet e fituara nga ky punim japin një kontribut të vlefshëm në aspektin e përcaktimit të përqendrimit të metaleve në tokë dhe translokimin e tyre në bimë, si dhe kontribut shkencor në njohjen e nivelit toksik që shkaktojnë te kjo bimë e kultivuar në këtë zonë. Bazuar në faktin se, bima e misrit është bioakumuluese dhe një ndër llojet kryesore të drithërave që kultivohet edhe tek ne, ky hulumtim do shërbejë si model për të vlerësuar toksicitetin e metaleve të rënda nëpërmjet përcjelljes së biomarkerëve specifik në bimë dhe jo vetëm. Prandaj rezultatet arritura, do të kontribuojnë drejtpërsëdrejti në njohjen e mekanizmave mbrojtës dhe ndërveprues të nivelit molekular në kuadër të qelizave bimore. Kontribut tjetër i rëndësishëm është se, rezultatet e këtij studimi janë të dobishme për të orientuar identifikimin e pikave të nxehta të akumulimit të metaleve të rënda në tokat bujqësore dhe ndikimet e drejtpërdrejta në cilësinë e tokës, por edhe mundësinë e bartjes në hallkat e tjera të zinxhirit ushqyes. Nga kjo do të përfitojnë drejtpërdrejtë edhe banorët lokal dhe institucionet përkatëse për të kuptuar gjendjen e saktë mjedisore të kësaj zone dhe hapat që duhet të ndërmerren në menaxhimin efektiv të këtyre tokave.

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[1] Lutei që ta plotësoni formularin dhe ta dërgoni të nënshkruar me postë elektronike.

Pjesë të punimit të botuara në formë kumtese:

1. Liridon Buqaj, Bekim Gashi, Metin Tuna (2023). DNA content and cell cycle on maize plant (*Zea mays* L.) under heavy metals stress. 14th International Conference of the French Society of Plant Biology – Plant Biology Europe. July 3 – 6, 2023, Marseille, France. P 152-153.
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Vendi, data dhe nënshkrimi

Në Prishtinë, 12.02.2024

Nënshkrimi B. G

Prof. Asoc. Dr. Bekim Gashi

V.V.

UNIVERSITETI I PRISHTINËS "HASAN PRISHTINA"
FAKULTETI I SHKENCAVE MATEMATIKE DHE NATYRORE
DEPARTAMENTI I BIOLOGJISË

Msc. Liridon H. Buqaj

Vlerësimi i tolerancës ndaj stresit oksidativ te misri (*Zea mays* L.) të shkaktuar nga ndotja e tokës me metale të rënda në zonën përreth shkritores së Ferronikelit në Drenas

PUNIMI I DOKTORATËS

Prishtinë, 2024

UNIVERSITY OF PRISHTINA "HASAN PRISHTINA"
FACULTY OF MATHEMATICAL AND NATURAL SCIENCES
DEPARTMENT OF BIOLOGY

Msc. Liridon H. Buqaj

Evaluation of tolerance to oxidative stress in maize (Zea mays L.) caused by soil contamination with heavy metals in the area near the Ferronikel smelter in Drenas

DOCTORAL THESIS

Prishtina, 2024

UNIVERSITETI I PRISHTINËS "HASAN PRISHTINA"
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DEPARTAMENTI I BIOLOGJISË

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PUNIMI I DOKTORATËS

Mentor: Prof. Asoc. Dr. Bekim Gashi

Prishtinë, 2024

REZYMEJA

Në Kosovë deri më tani janë konfirmuar disa "pika të nxehta" mjedisore që karakterizohen me shkallë relativisht të lartë të ndotjes së komponenteve mjedisore, kryesisht si rezultat i aktiviteteve të ndryshme industriale. Në kontekst të kësaj e tillë është zona rreth shkritorës së "Ferronikelit" në Drenas si një ndër gjigantët eksploatues dhe përpunues sidomos të nikelit e cila vazhdon të mbetet një vatër me potencial aktiv të emetimit të ndotësve në mjedis gjë që paraqet një rrezik që prek pothuajse të gjitha ekosistemet pa dallim. Ekosistemet e tilla përreth burimeve të ndotjës në kuadër të tyre përmbajnë mes tjerash edhe përqendrime relativisht të larta të metaleve të rënda. Substancat e tilla kimike apo më konkretisht metalet e rënda shoqërohen me efekte të ndryshme të karakterit toksik në bimë në aspektin biokimiko-fiziologjik, gjenetik por edhe atakojnë proceset e ndryshme metabolike në kuadër të biotave. Prandaj, ky studim synon të hulumtojë shkallën e rrezikut të ndotjes të tokave bujqësore me metale të rënda (Fe, Cu, Mn, Cr, Cd, Ni dhe Pb) nga shkritorja e Ferronikelit në Drenas, dhe efektet e tyre toksike të mundshme në bimët e misrit, një kulturë ushqimore e ekspozuar ndaj ekspozimit të zgjatur gjatë gjithë sezonit të rritjes. Objektiv tjetër i këtijë studimi është edhe trajtimi i bimëve të misrit me përqendrime të ndryshme të Ni, Cr dhe Pb, me çka ne synojmë të parashikojmë efektet e dëmshme të mundshme në bimë që vijnë nga përqendrimet e ndryshme të këtyre metaleve, duke përcaktuar kështu kufijtë e tolerancës së tyre toksike për referencë në të ardhmen. Për të arritur këtë objektiv, bimët e misrit u rritën në tokë të mbledhur pranë shkritores së Ferronikelit në Drenas në Kosovë, dhe u trajtuan me përqendrime të ndryshme të Ni dhe Cr (50, 100, 200, dhe 400 ppm), dhe Pb (20, 50 , 100 dhe 200 ppm). Biomarkerët përfaqësues të zhvilluar për këtë qëllim u përdorën për të vlerësuar shkallën e stresit oksidativ qelizor për shkak të toksicitetit të mundshëm të metaleve dhe ky hulumtim mendojmë se është i pari i këtij lloji në këtë fushë sepse pasqyron qartë efektet e kësaj ndotjeje në organizmat e gjallë. Për të vlerësuar shkallën e efekteve toksike dhe stresit oksidativ të shkaktuar nga këto metale të rënda në bimët e misrit, ne përdorëm biomarkues të ndjeshëm, duke përfshirë aktivitetin e acidit dehidratazë δ-aminolevulinik (ALA-D), përmbajtjen e acidit δ-aminolevulinik (ALA), përmbajtjen e klorofilit, glutationin. Nivelet (GSH), peroksidimi i lipideve (MDA), si dhe duke vlerësuar përmbajtjen e ADN-së dhe dinamikën e ciklit qelizor. Sipas rezultateve të hulumtimit tonë përqendrimi i metaleve të rënda në dheun dhe në gjethet e misrit u konstatuan pranë shkritores së Ferronikelit, dhe atë në disa lokalitete, përqendrimi i Ni dhe Cr tejkaloi 800 mg kg^{-1}. Një efekt i rëndësishëm i toksicitetit të shkaktuar nga metalet e rënda rezultoi në akumulimin e ALA-së dhe

zvogëlimin e aktivitetit të D-ALA-së dhe përmbajtjen e klorofilit në gjethet e misrit. Në përgjithësi, metalet e rënda ndikuan edhe në sasinë e ADN-së bërthamore dhe në fazat e ciklit qelizor (G1, S, G2). Ndryshime në shkallë sinjifikante në sasinë e ADN-së u konstatuan midis lokaliteteve të hulumtuara. Gjithashtu, dallime sinjifikante u konstatuan në dinamikën e ciklit qelizor, veçanërisht në fazat G1 dhe G2, ndërsa në fazën S nuk u gjetën dallime sinjifikante. Nën ndikimin e metaleve të rënda, përgjithësisht vërehet një shkallë më e ulët e shpërndarjes së qelizave në fazën G1 dhe një shkallë e rritur e shpërndarjes së qelizave në fazën G2. Kjo mund të konsiderohet si një strategji mbrojtëse për të eliminuar gabimet në materialin gjenetik me mekanizma riparimi apo edhe si një hap drejt vdekjes së programuar gjenetike. Në anën tjetër, gjetjet tona për intoksikimin laboratorik tregojnë se bimët e misrit kanë akumuluar përqendrim dukshëm më të lartë Ni dhe Cr në gjethet e tyre kur ato janë shtuar në tokë krahasuar me kontrollin, dhe bashkërendimi i tyre korrespondon me përqendrimin e shtuara në tokë. Për më tepër, ekzistojnë disa marrëdhënie sinergjike midis këtyre metaleve dhe përqendrimit të Fe dhe Mn në gjethe. Megjithatë, ky akumulim i metaleve të rënda, veçanërisht Ni dhe Cr në 400 ppm, rezulton të ketë efekte të konsiderueshme negative në aktivitetin e D-ALA-së, me inhibim deri në 50% dhe ulje të ndjeshme të përmbajtjes totale të klorofilit, krahasuar me bimët e kontrollit. Për më tepër, rezultatet treguan se Ni dhe Cr në përqendrim më të larta të aplikuar në tokë ishin edhe arsyeja e rritjes së niveleve të ALA, GSH dhe MDA në gjethet e misrit, krahasuar me grupin e kontrollit. Stresi i metaleve të rënda ndikoi ndjeshëm në dinamikën e ciklit qelizor, veçanërisht në përqendrime më të larta të Ni dhe Cr, ku fazat G1 dhe G2/M ishin pothuajse të barabarta. Kjo tregon një ndalim të theksuar të ciklit qelizor dhe nënvizon efektet e dëmshme të këtyre metaleve në nivelin e ADN-së. Bima e misrit, si një kulturë e zakonshme në mbarë botën, mund shërbej si një model i përshtatshëm për studimin e toksicitetit të mundshëm që rezulton nga ndotja e tokave bujqësore me metale të rënda. Ky hulumtim kontribuon në të kuptuarit tonë për menaxhimin dhe zbutjen e ndotjes me metale të rënda në zonat bujqësore dhe implikimet e tij të mundshme për mekanizmat mbrojtës të bimëve.

Fjalë kyçe: Ferronikel, metale të rënda, misri, biomarkerët, stresi oksodativ qelizor, sasia e ADN bërthamore, cikli qelizor.

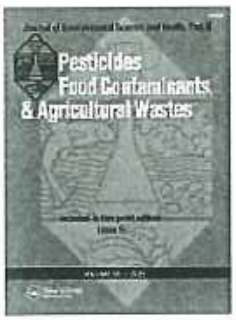
SUMMARY

In Kosovo, several environmental "hot spots" have been identified, characterized by a relatively high level of pollution in environmental components, primarily due to various industrial activities. One such area is around the "Ferronickel" smelter in Drenas, which is a significant mining and processing facility, particularly for nickel. This area remains a concern due to its potential for emitting pollutants into the environment, posing risks to nearly all ecosystems. Ecosystems surrounding pollution sources often contain relatively high concentrations of heavy metals. These chemical substances, specifically heavy metals, can have various toxic effects on plants, impacting biochemical, physiological, and genetic processes. They can also disrupt metabolic processes within the biota, further highlighting the environmental risks associated with industrial pollution in Kosovo. This study aims to investigate the extent of the risk of agricultural soil contamination with heavy metals (Fe, Cu, Mn, Cr, Cd, Ni, and Pb) from the Ferronikel smelter in Drenas, Kosovo, and its potential toxic effects on maize plants, a food crop exposed to prolonged exposure throughout the growing season. Another objective of this study is the treatment of maize plants with different concentrations of Ni, Cr and Pb, which additionally we aim to forecast potential harmful effects on plants resulting from various concentrations of these metals, thus determining their toxic tolerance limits for future reference. To achieve this objective, maize seedlings were grown in soil collected near the Ferronikel smelter in Drenas in Kosovo, and treated with salt solution of different concentrations of Ni and Cr (50, 100, 200, and 400 ppm) and Pb (20, 50, 100, and 200 ppm). Representative biomarkers developed for this purpose were used to assess the extent of cellular oxidative stress due to the potential toxicity of the metals and this research is the first of its kind in this area because it clearly reflects the effects of this pollution on living organisms. To assess the extent of toxic effects and oxidative stress induced by these heavy metals on maize plants, we used sensitive biomarkers, including δ-aminolevulinic acid dehydratase (ALA-D) activity, δ-aminolevulinic acid (ALA) content, chlorophyll content, glutathione (GSH) levels, lipid peroxidation (MDA), as well as by evaluating DNA content and cell cycle dynamics. The highest concentrations of heavy metals in soils and maize leaves were conducted close to the Ferronikel smelter, and in some locations, the Ni and Cr concertation in soils exceeded 800 mg kg^{-1}. A significant effects of heavy metals induced toxicity resulted in the, build-up aminolevulinic acid and reduced activity of δ-aminolevulinic acid dehydratase, and chlorophyll content in the maize

leaves. In general, heavy metals affect the amount of nuclear DNA and the phases of the cell cycle (G1, S, G2). Significant differences in DNA content were found between the analyzed groups of maize leaves. Particularly, significant differences are observed in the dynamics of the cell cycle, especially in the G1 and G2 phases, while no significant differences are observed in the S phase. Under the influence of heavy metals, a lower rate of cell distribution in G1 phase and an increased rate of cell distribution in G2 phase are generally observed. This can be considered as a protective strategy to eliminate errors in the genetic material by repair mechanisms or even as a step towards programmed genetic death.

On the other hand, our findings for laboratory intoxication, indicate that maize seedlings have accumulated significantly higher concentration Ni and Cr in their leaves where have applied each other in soil compared to control, and their concertation corresponding with applied concentration in the soil. Furthermore, there are some synergistic relations between these metals and Fe and Mn concentration in leaves. However, this accumulation of heavy metals, especially Ni and Cr at 400 ppm, resulting to have significantly negative effects in ALA-D activity, with inhibition up to 50% and significantly decrease total chlorophyll content, compared to control plants. Moreover, results showed that Ni and Cr in higher concentration applied in the soil were the reason also of increasing in ALA, GSH and MDA levels in leaves of maize seedlings, compared with control group. Heavy metal stress significantly affected cell cycle dynamics, especially at higher concentrations of Ni and Cr, where the G1 and G2/M phases were nearly equal. This indicates a pronounced arrest of the cell cycle and underscores the harmful effects of these metals on the DNA level. The maize plant, a common crop worldwide, serves as a suitable model for studying the potential toxicity resulting from contamination of agricultural soils with heavy metals. This research contributes to our understanding of managing and mitigating heavy metal contamination in agricultural areas and its potential implications for plant defense mechanisms.

Key words: Ferronikel, heavy metals, maize, biomarkers, oxidative stress level, nuclear DNA content, cell cycle.



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Stress induced by soil contamination with heavy metals and their effects on some biomarkers and DNA damage in maize plants at the vicinity of Ferronikel smelter in Drenas, Kosovo

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ABSTRACT

The Ferronikel smelter in Drenas is one of the main industrial areas in the Kosovo and pollution by heavy metals causes serious threat for all living organisms on this area. The objective of this study was to determine the concentration of some heavy metals (Fe, Cu, Mn, Cr, Cd, Ni and Pb) in agricultural soils and in maize plants, and their potential toxic effects on this plant through some sensitive biochemical and molecular markers. Maize seedlings growth in nine soil samples from different locations of this area. The highest concentrations of heavy metals in soils and maize leaves were conducted close to the Ferronikel smelter, and in some locations, the nickel and chromium concertation in soils exceeded 800 mg kg^{-1}. A significant effects of heavy metals induced toxicity resulted in the, build-up aminolevulinic acid and reduced activity of δ-aminolevulinic acid dehydratase, and chlorophyll content in the maize leaves. In general, maize seedlings growth in polluted locations showed an increase in nuclear DNA content and in G2M phase. We concluded that locations close to the smelter are affected by soil heavy metals pollution and these biochemical and molecular analysis would be a powerful ecotoxicological tool in biomonitoring of heavy metal pollution.

KEYWORDS

Heavy metals; soil; maize; biomarkers; DNA content; cell cycle

Introduction

Heavy metals are considered environmental pollutants due to their high toxic potential, their ability to accumulate in various environmental components, and their persistence in the environment.[1] Contamination of terrestrial and aquatic ecosystems with heavy metals is an environmental problem and has public health implications. These elements penetrate into various environmental components such as water, soil, and air and pose a threat to living organisms.[2] From a chemical point of view, heavy metals include certain metals and metalloids with a density greater than 5 g cm^{-3}. Some of them are essential micronutrients, but they have specific mechanisms of action characterized by high affinity for sulfhydryl groups, competition with essential elements, and generation of oxidative stress.[3] Heavy metals are accumulated in soil as a result of various industrial activities, leading to elevated concentrations near such areas, often exceeding permissible limits. Therefore, it is important to systematically monitor these areas and measure the concentrations of metal ions.[4] Plants grown in these conditions pose potential risks to human health when consumed.[5]

Several studies have been conducted in Kosovo to determine heavy metal concentrations in soil samples collected near industrial areas.[6–8] A study conducted in Kosovo covered two regions, Mitrovica and Drenas, and provided data on metal concentrations in agricultural soils and their accumulation in different crops, such as wheat, maize, potatoes and grass by measuring concentrations of heavy metals, including Cd, Cr, Cu, Ni, Pb, and Zn, separately in vegetative and reproductive parts.[9]

Plants growing in substrates containing varying amounts of heavy metals suffer from stress, resulting in lower productivity and potential toxicity. However, plants have complex counter mechanisms at different levels, including molecular, biochemical-physiological, and cellular responses, to cope with these stresses.[10] According to Rai et al.,[11] heavy metals affect various metabolic processes, with a focus on photosynthesis. They can interfere with various steps of the chlorophyll synthesis pathway and affect the enzymatic system involved in this process, leading to the formation of reactive oxygen species (ROS), effects on the electron transport system in the photosynthetic apparatus, lipid peroxidation, and replacement of the Mg ion in the core of the pyrrole rings. Chlorophyll

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biosynthesis is an important physiological process that occurs through a series of reactions that produce intermediates until the final step of chlorophyll synthesis occurs. Specific enzymes are usually involved in these reactions. In general, two main groups of reactions are distinguished: the ALA synthesis and the conversion of protoporphyrin IX to chlorophyll. [12] ALA serves as a common precursor in the synthesis of tetrapyrrole and is converted to porphobilinogen-PGB by the enzyme delta-aminolevulinic acid dehydratase (ALA-D). ALA Dehydratase is a type of metal enzyme that requires the presence of Mg ions for its activation in plants.[13] It has been suggested that high concentrations of certain heavy metals have a direct effect on ALA-D activity under both "*in vivo*" and "*in vitro*" conditions.[14] The accumulation of ALA in various plant parts, such as nodes, leaves, and roots, is the result of the inhibition of the activity of this enzyme. This accumulation promotes the formation of reactive oxygen species and increases cellular oxidative stress. Consequently, the activity of antioxidant enzymes increases to scavenge these reactive oxygen species.[15] Therefore, it is crucial to analyze the activity of ALA -D in relation to different types of metals. Several authors have provided such data by studying different plant species grown in polluted areas,[8] or by laboratory experiments with different doses of heavy metals for poisoning purposes.[16,17]

To mitigate the harmful effects of heavy metals, plants activate enzymatic and non-enzymatic mechanisms that make them less harmful to cells. Under these conditions, the activity of antioxidant enzymes (superoxide dismutase, catalase) and ascorbic acid increases, while malondialdehyde levels, indicators of lipid peroxidation, also increase. In addition, photosynthetic process and chlorophyll content decrease in response to heavy metal concentration.[18] Assessment of oxidative stress in plants may include measuring the degree of lipid peroxidation by the product malondialdehyde. Results show an increase in malondialdehyde levels as a function of heavy metal concentration, as well as changes in chlorophyll, carotenoid, and proline levels as a function of heavy metal treatment duration and concentration.[19] As a result of exposure to heavy metals, plants experience oxidative stress that causes cellular damage and disrupts cellular ionic balance. In response, plants activate protective mechanisms, including the production of reduced glutathione (GSH), an antioxidant that protects cells from free radicals. Another mechanism is the synthesis of phytochelatins, cysteine-rich peptides produced enzymatically from reduced glutathione by phytochelatin synthetase.[20] Measurement of various biomarkers in plants provides insight into the mechanisms by which plants combat environmental stress. These biomarkers can be specific or general indicators of one or more environmental stressors.[21] Chlorophyll content can also serve as a biomarker for monitoring the effects of various metals, as a decrease in chlorophyll content is observed in response to heavy metal concentration.[22] The presence of heavy metals in the environment generates free radicals and affects the internal enzymatic and non-enzymatic antioxidant mechanisms in plants.[23] For example, *Andrographis paniculata* exhibits visible changes in total antioxidant capacity (TAC) values depending on the concentration of metals such as Cu, Co and Sn.[24]

In addition, studies have shown the genotoxic effects of heavy metals in plants, with significant effects on DNA nuclear content.[25] The analysis of the distribution of DNA content (genome size) and cell cycle phases (G1, S and G2) is scientifically relevant for the species studied. Flow cytometry is a rapid method for determining nuclear DNA content (2cDNA) and analyzing the phases of the cell division cycle. This technique is widely used in various biological disciplines and it is becoming more and more refined analytically.[26] Areas contaminated with heavy metals affect intraspecific genome size (DNA content within a species), chromosome number, and cell cycle.[27]

The main hypothesis of this work is that translocation of heavy metals from soil to plants affects the main biochemical process pathway of chlorophyll synthesis, it induces oxidative stress, and it has molecular effects on DNA content and cell cycle in maize plants in response to heavy metal stress.

This study aims to investigate the extent of the risk of agricultural soil contamination with heavy metals (Fe, Cu, Mn, Cr, Cd, Ni, and Pb) from the Ferronikel smelter in Drenas, Kosovo, and its potential toxic effects on maize plants, a food crop exposed to prolonged exposure throughout the growing season. Representative biomarkers developed for this purpose were used to assess the extent of cellular oxidative stress due to the potential toxicity of the metals and this research is the first of its kind in this area because it clearly reflects the effects of this pollution on living organisms. The stress response of maize plants to these pollutants was evaluated by measuring various biochemical and physiological parameters (ALA-D activity, ALA content, total chlorophyll content, GSH, MDA, TAC) as well as molecular parameters (DNA nuclear content, cell cycle phases) in maize plants.

Material and methods

Soil samples were collected from agricultural soils in nine localities in the vicinity of the Ferronikel smelter in the municipality of Drenas, Kosovo (L1–L9). A control location in Budakovë village, Suharekë municipality, Kosovo, was also included. Soil sampling and maize seeding locations are indicated on the map (Fig. 1).

Soil sampling involved taking samples at 30–40 locations within a 50-m radius. Hand augers were used to collect soil samples at a depth of 0–30 cm at each location. Sampling was done randomly according to the guidelines of BBodSchV, Theocharopoulos et al. and ICP Forest.[28–30] Each soil sample was divided into two groups: one group was used for chemical composition analysis and the other group was for maize seeding.

The maize plants were cultivated in a vegetative room for about four weeks until they reached the 3–5 leaf stage. The leaves of *Zea mays* plants were then used for chemical analysis to determine the metal concentrations and for the evaluation of the biochemical and molecular parameters studied.

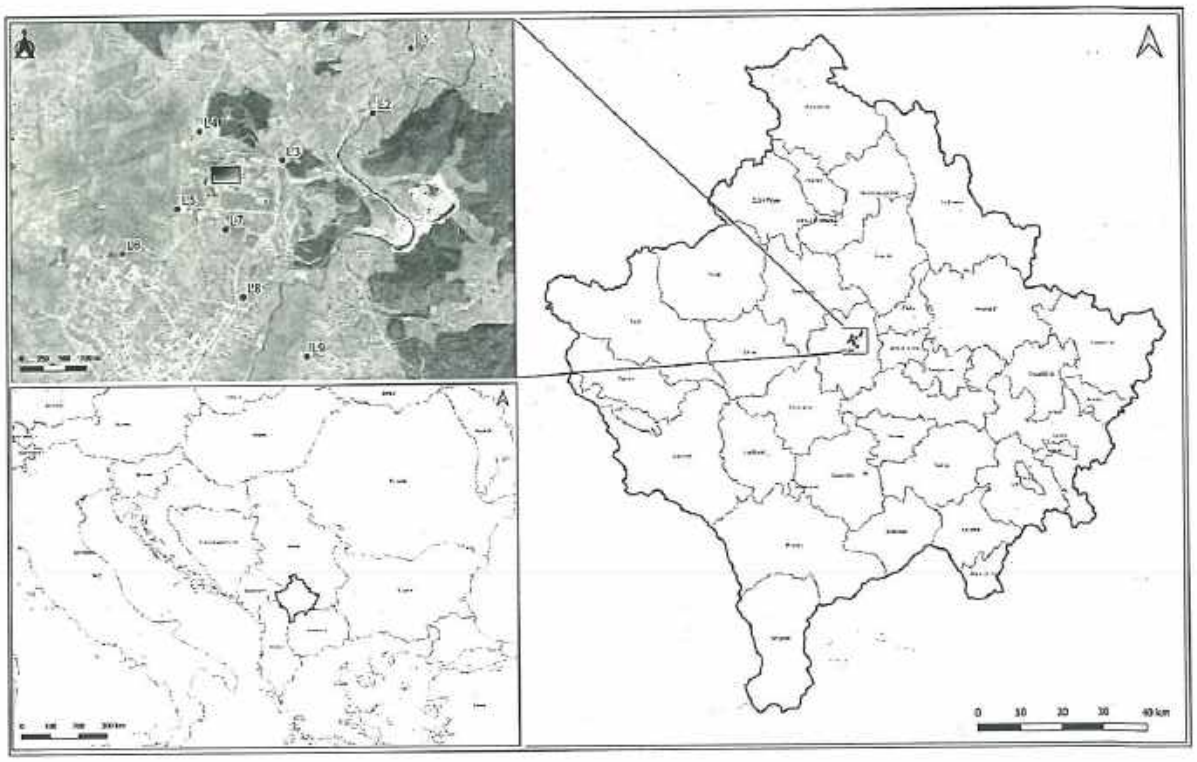

Figure 1. Map of the locations of sampling points in Drenas, Kosovo: L1–L9 near the smelter Feronikel in Drenas, Kosovo and the control site, Budakovë, Suharekë, Kosovo. Source: Dardan Hoti, GIS expert, Kujtesa Prishtine, Kosovo.

Soil properties

Soil pH was measured in a suspension of H_2O and 0.01 M $CaCl_2$ at a ratio of 1:2.5 according to the guidelines of DIN ISO 10390.[31] The amount of soil organic carbon and total nitrogen (N) were determined by the following procedure: 1 g of the dried fine soil sample was mixed with 10 mL of 0.1667 M potassium dichromate and 20 mL of 36% sulfuric acid. After 30 min, 250 mL of distilled water was added followed by 3–4 drops of the indicator solution. Titration was performed with a 1 M ferrous sulfate solution according to the method of Walkley-Black.[32]

Soil texture was determined using the following protocol: 10 g of a dried soil sample was taken and then 25 mL of 10% HCl was added to dissolve the carbonates. Then, 15 mL of 30% H_2O_2 was added to decompose the organics, and the mixture was combined with 20 mL of pyrophosphate solution ($Na_4P_2O_7$ x 10 H_2O) in a 250 mL plastic container. The container was placed on a stirring machine set at 50 rpm and left overnight. The next day, the sand fraction was separated directly with a sieve, while the silt and clay fractions were determined based on Stock's law, in which the settling of soil particles in a liquid environment is observed. The silt fraction was determined with a pipette at a depth of 10 cm, while the clay fraction was determined at a depth of 5 cm. The suspension was then dried at 105 °C for 24 h and the percentage of silt and clay was calculated.[33]

Chemical analyses

The soil samples were air dried to complete dryness and then mechanically ground. The sieved material with grain size less than <2mm was used for analysis. The leaves of the maize plants were rinsed with deionized water after 28 days and placed in a dryer at 105 °C for about 24 h. The dried material was then crushed, sieved and used for chemical analysis. The analysis of the soil samples was carried out at the Agricultural Institute of Kosovo in Pejë, Kosovo, according to this procedure: 0.3 g of a well-mixed, dried, crushed, and sieved soil sample was placed in a Teflon vessel, and the weight was measured to the nearest 0.001 g. In a fume hood, 0.1 mL of H_2O_2, 10 mL of concentrated nitric acid, and 3 mL of concentrated hydrochloric acid were added to the Teflon vessel, which was equipped with a controlled pressure mechanism. The soil samples were analyzed using the instrument MP-AS. Chemical analysis of maize leaves was performed in the analytical laboratory "Agrovet" in Fushë Kosovë, Kosovo, according to this procedure: Leaf samples weighing 500 mg were placed in a Teflon vessel and 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added. Digestion was performed in a "Berghof" type microwave oven. Leaf samples were analyzed using an ICP-OES instrument.

Biochemical analyses

δ-aminolevulinic acid dehydratase (ALA-D) activity

The enzyme was determined colorimetrically using a modified Ehrlich reagent to determine the amount of PBG (porphobilinogen) formed. Extraction and assay of ALA-D was performed according to the procedure described in Jain and Gadre[34] and slightly modified by Osmani et al.[35] The enzyme was extracted using ice-cold 50 mM Tris-HCl buffer (pH 8.4) containing 0.2% Triton X100. Leaves of similar size

were taken from ten plants and each of them (500 mg) was ground to a fine powder in a mortar at ice-cold temperature. Immediately after grinding, 1 g of polyvinylpolypyrrolidone was mixed with the powdered tissue to prevent oxidation of phenols. After filtration, the homogenate was centrifuged at 15 000 g for 20 min at 4°C. The pellet was discarded, and the supernatant was used as the enzyme source. One mL of enzyme was incubated with 0.27 mL of 1 mg mL^{-1} ALA, 1.35 mL of 50 mM Tris-HCl buffer (pH 8.5) and 0.08 mL of 0.02 M $MgCl_2$. The reaction was initiated by adding the extract at time zero. After 1 h of gentle shaking (150 rpm) at 37°C, the reaction was stopped by adding 0.3 mL of 3 M TCA, followed by centrifugation at 5,000 g for 10 min. For PBG estimation, the supernatant was mixed with Ehrlich reagent (prepared fresh by dissolving 1 g of 4-dimethyl aminobenzaldehyde in 30 mL of glacial acetic acid and 8 mL of 70% PCA, and then made up to 50 mL with glacial acetic acid) in a ratio of 1:1 (v/v). Absorbance was measured at 553 nm after 15 min against zero time control. One unit of enzyme activity was defined as 1 nmol of PBG formed per hour. Protein content was determined according to the Bradford method.[36]

Aminolevulinic acid (ALA) content

This was determined according to the method of Tewari & Tripathy.[37] Leaves of similar size were taken from ten plants and were homogenized using 1 mL of 1 M Na-acetate buffer (pH 4.6). After centrifugation at 15,000 × for 15 min at 4°C, ALA of the supernatant was condensed with ethyl acetoacetate to give PBG: 0.7 mL supernatant, 0.8 mL distilled water and 0.1 mL ethylacetoacetate mixture were placed in a boiling water bath for 10 min.[35] After cooling, an equal volume of Ehrlich reagent was added and the colored complex formed was read for absorbance at 553 nm. The amount of PBG formed was calculated using a standard curve from ALA and the results were expressed in μM ALA/g leaf fresh weight (FW).

Total chlorophyll determination

Chlorophyll was extracted from fresh leaves (100 mg) of maize using 80% acetone. Chlorophyll content was calculated using absorbance values at 663 nm and 645 nm measured with a UV–Vis spectrophotometer. The new extinction coefficients and the reevaluated equations of Lichtenthaler were used.[38] Total chlorophyll content was expressed as mg g^{-1} leaf dry weight (DW).

Glutathione (GSH)

Fresh leaves from maize plant were homogenized with 5% TCA. The homogenate was centrifuged at 15,000 × g for 20 min, and the pH of the supernatant was adjusted to 4.0–5.0 with 1 M NaOH. The content of glutathione (GSH) in the crude extract was determined using the Ellmann method [DTNB: 5,5-dithiobis (2-nitrobenzoic acid)],[39] in which the reaction mixture comprised 0.1 mL of sample, 2 mL of 100 mM pH 8.4 Tris–HCl buffer and 0.1 mL Ellmann reagent (60 mg/100 mL Tris–HCl buffer 0.1 M, pH 7.0). The absorbance of the reaction mixture was read at 412 nm. Glutathione concentration in the samples was calculated using the standard curve for GSH. Data were expressed as μM g^{-1} fresh weight of leaf.

Malondialdehyde (MDA) assay

Lipid peroxidation was estimated by determining the content of malondialdehyde (MDA) in leaves according to the method of Hodges et al.[40] Fresh leaves (500 mg) of maize plant were ground in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 x g for 20 min at 4°C. MDA content was determined by the corrected thiobarbituric acid (TBA) method. Two milliliters of extraction solution and 3 mL 0.5% TBA in 10% TCA were mixed vigorously. The mixture was heated at 95°C in a constant temperature water bath for 30 min and then cooled on ice to room temperature. After centrifugation at 5,000 × g for 10 min, the supernatant absorbance at 450, 532 and 600 nm was measured using a UV–vis spectrophotometer. The MDA concentration was determined using the formula CMDA (μmol mL^{-1}) = 6.45 x (D532–D600) − 0.56 x D450, where D450, D532 and D600 are the absorbencies at 450, 532 and 600 nm, respectively. Data were expressed as μM g^{-1} fresh weight of leaf.

Total antioxidant capacity (TAC)

the total antioxidant capacity of *Z. mays* L. leaf extracts was determined by the phosphomolibdium method.[41] The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analysis and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1 mL of the extracts (1 mg mL^{-1}) was mixed in an Eppendorf tube with 1 mL of the reagent solution (0.6 M H_2SO_4, 28 mM NaH_2PO_4, 4 mM $(NH_4)_6Mo_7O_{24}$ $4H_2O$). The tubes were sealed and incubated in a thermoblock at 95°C for 90 min. After the samples cooled to room temperature, the absorbance of the aqueous solution was measured at 695 nm against a blank. The TAC was determined by comparison with the ascorbic acid standard calibration curve. The experiment was performed in triplets and values are expressed as equivalents of ascorbic acid. Data were expressed as μM mg^{-1} fresh weight of leaf.

Molecular analyses

Genomic size (nuclear DNA content) and cell cycle determination

analysis of nuclear DNA content on maize leaves was performed according to the procedures described by Tuna et al.[42] Suspensions of intact nuclei were prepared using commercial kits from Partec (Munster, Germany). The fresh leaf tissue of each sample (20 mg) and a standard leaf tissue (Secale cereale, 20 mg) were simultaneously minced in a Petri dish containing 0.5 ml of extraction buffer. The

homogenized solution was transferred through a 30 µm filter into a glass tube, and then 2 ml of staining buffer (CyStain PI absolute P) was added to each tube. Prior to analysis, samples were incubated at room temperature in the dark for at least 30 min. Fluorescence intensities of the stained nuclei were measured using a Partec CyFlow Space flow cytometer (Munster, Germany), and the results were analyzed using FloMax analysis software developed specifically for this cytometer. The 2C nuclear DNA content of the samples was calculated based on the relative positions of the G1 peaks of the sample and the standard. The G1, S and G2M phases of the interphase of the cell cycle are determined using the flow cytometer according to the procedure described above. For this purpose, only the leaves of the maize plant are homogenized, but not those of the standard plant.

Data analysis

The experiment was performed in a randomized design with three replicates. Differences between locations and among parameters were tested using SPSS 17 statistical program. Statistical variance analysis of all data were performed using one-way ANOVA, and mean comparison was performed with Duncan Multiple Range Test at the 5% level of significance.

Results and discussion

The data on the physicochemical properties of the soil, including the pH, the percentage of soil organic carbon (C%), the total nitrogen (N%) and the soil texture are presented in Table 1. The highest value of pH(H_2O) = 7.97 was found in locality five (L5), while pH($CaCl_2$) = 7.70 was recorded in locality control. The lowest value of pH(H_2O)= 6.61 and pH($CaCl_2$)-5.82 was found at the sixth locality (L6). The highest content of organic matter was measured in the frame of the second location (L2) = 4.74%, while the lowest value was recorded in the frame of the third location (L3) = 1.01%. The highest content of total nitrogen was measured in the frame of the fifth location (L5) = 0.50%, while the lowest value was measured in the frame of the third location (L3) = 0.05%. As for soil texture, the highest values were measured for sand at the fourth location (L4) = 27.59%, for silt at the second location (L2) = 40.76%, and for clay in locality one (L1) = 48.36%. The lowest values for sand were found in locality one (L1) = 11.29%, for silt in locality six (L6) = 32.24%, and for clay in the control locality 36.44%.

Table 1. Physicochemical properties of the soil at experimental sites.

					Soil texture %			
Sites	pH (H2O)	pH (CaCl2)	C%	N%	Sand	Silt	Clay	Texture name (class)
Control	7.87	7.70	3.47	0.19	24.53	39.03	36.44	Clay loam (CL)
L1	6.86	6.45	1.10	0.07	11.29	40.35	48.36	Silty clay (SC)
L2	7.15	6.70	4.74	0.26	18.59	40.76	40.65	Silty clay (SC)
L3	7.75	7.17	1.01	0.05	19.10	32.62	48.28	Clay (C)
L4	7.91	7.43	2.80	0.15	27.59	33.55	38.86	Clay loam (CL)
L5	7.97	7.38	3.14	0.50	18.48	34.87	46.65	Clay (C)
L6	6.61	5.82	1.55	0.08	19.98	32.24	47.78	Clay (C)
L7	7.49	7.03	3.59	0.20	17.69	35.14	47.17	Clay (C)
L8	7.35	6.77	1.37	0.08	18.01	37.76	44.23	Clay (C)
L9	7.35	6.91	3.27	0.18	18.74	33.49	47.77	Clay (C)

The physicochemical properties of soil play an important role in the uptake of heavy metals by plants through their root systems. According to Takáč et al.,[43] an increase in pH, organic matter content, and clay content leads to a decrease in the uptake capacity of plants for heavy metals.

The results of heavy metal concentrations (Fe, Cu, Mn, Cd, Ni, Cr, and Pb) in soil samples and maize plant leaves are presented in Table 2. Our results indicate higher concentrations of all mentioned elements in nine localities around Ferronikeli smelter in Drenas, compared to the control group, village Budakovë in municipality, Suharekë, Kosovo. Location one (L1) had the highest concentrations of Cu (64.19 mg kg^{-1}), Mn (1,666.01 mg kg^{-1}), and Cd (2.91 mg kg^{-1}). Location two (L2) had the highest concentrations of Fe (41,109.43 mg kg^{-1}), Cr (801.09 mg kg^{-1}), and Ni (809.46 mg kg^{-1}), while location seven (L7) had the highest concentration of Pb (59.90 mg kg^{-1}).

Furthermore, the higher concentration of heavy metals, especially nickel and chromium with ten or more times higher was conducted in localities vicinity to smelter or at waste collection points of Feronikeli smelter. These results indicate that agricultural soils which are surrounded to smelter have potentially harmful effects for all living organisms. Among other metals, Ni, Cr and Cd, were in higher concentration in the locations in the direction of east from Ferronikel smelter, which is related to waste industrial collection of the smelter and mostly the direction of the wind. In general, the data from other studies indicate higher concentrations of heavy metals in industrial areas exceeding the permissible standards.[4] The concentrations of Ni (258.54 mg kg^{-1}) and Cr (203.22 mg kg^{-1}) in agricultural areas near Ferronikel smelter were reported by Zogaj and During.[9] Several studies conducted by different authors in the case of Kosovo [6,7] clearly demonstrate an increase in the concentration of these elements in the vicinity of industrial areas, posing a potential risk to the environment. A study conducted by Zogaj et al.[44] provides data on concentrations of some heavy metals in agricultural soils near industries, including Pb (15.6–2,206.3 mg kg^{-1}), Ni (12.5–2,864 mg kg^{-1}), Cd (0.036–14.16 mg kg^{-1}), Cu (9.36–92.65 mg kg^{-1}), and Cr (17.3–1,444.7 mg kg^{-1}). Our results are within the range of these previously reported values.

As for the concentration of the metals in the leaves of the maize plant, our studies gave the following results, which are presented in Table 3. Concentrations of Cd and Pb in all maize leaf samples were under limit of the detection by instrument ICP-OES. Nickel (Ni) was detected only in leaf samples from location one (L1) at a concentration of 1.97 mg kg^{-1} and from location two (L2) at a concentration of 0.71 mg kg^{-1}, which corresponds to the higher Ni concentration in the soil of these locations. The highest concentrations of Fe (72.45 mg kg^{-1}) and Cu (5.89 mg kg^{-1}) in the leaf samples were found in plants from location one (L1). Location two (L2) had the highest concentration of Cr in the leaves (1.73 mg kg^{-1}), while the control samples had a Cr

Table 2. Statistical data of Fe, Cu, Mn, Cr, Cd, Ni and Pb concentrations (mg kg^{-1}) in soil samples from some polluted locations of Drenas, Kosovo (L1–L9) and non-polluted location of Budakovë (control site), Suharekë, Kosovo.

		Soil samples – heavy metals						
		Fe	Cu	Mn	Cr	Cd	Ni	Pb
Non-polluted region	**Control group**	**19,166.24^{I}** ±336.81	**16.51^{E}** ±0.31	**1,551.69^{B}** ±41.83	**27.92^{H}** ±0.74	**0.10^{G}** ±0.05	**20.39^{I}** ±3.43	**44.36^{E}** ±0.52
Polluted regions	**L1**	**35,892.81^{C}** ±97.18	**64.19^{A}** ±3.78	**1,666.01^{A}** ±28.85	**373.06^{B}** ±11.86	**2.91^{A}** ±0.08	**312.84^{C}** ±3.12	**54.75^{B}** ±1.30
	L2	**41,109.43^{A}** ±381.31	**30.78^{D}** ±2.97	**1,155.55^{D}** ±34.23	**801.09^{A}** ±27.94	**0.47^{F}** ±0.02	**809.46^{A}** ±8.06	**55.98^{B}** ±0.38
	L3	**36,840.99^{B}** ±110.77	**25.12^{C}** ±0.29	**1,141.66^{D}** ±30.11	**327.99^{C}** ±8.50	**0.33^{FG}** ±0.05	**425.42^{B}** ±9.24	**41.89^{F}** ±0.31
	L4	**25,148.15^{G}** ±478.67	**19.98^{C-E}** ±0.09	**822.28^{E}** ±29.59	**150.82^{EF}** ±0.75	**0.54^{F}** ±0.13	**142.31^{F}** ±1.02	**50.75^{C}** ±0.23
	L5	**31,482.24^{D}** ±79.93	**23.88^{CD}** ±1.22	**1,283.67^{C}** ±52.25	**229.85^{D}** ±3.47	**0.90^{E}** ±0.11	**266.19^{D}** ±18.15	**59.79^{A}** ±0.90
	L6	**25,019.93^{G}** ±42.57	**19.22^{DE}** ±0.47	**880.57^{E}** ±12.71	**100.54^{G}** ±1.12	**1.55^{D}** ±0.05	**67.69^{H}** ±0.87	**48.12^{D}** ±0.27
	L7	**28,894.28^{E}** ±157.61	**20.93^{C-E}** ±0.34	**1,261.35^{C}** ±37.98	**170.06^{E}** ±3.73	**2.12^{C}** ±0.13	**243.82^{E}** ±1.61	**59.90^{A}** ±0.65
	L8	**27,190.35^{F}** ±436.19	**23.12^{CD}** ±1.73	**1,098.84^{D}** ±13.62	**132.05^{F}** ±4.73	**2.24^{BC}** ±0.15	**108.27^{G}** ±1.27	**49.69^{CD}** ±0.81
	L9	**20,632.26^{H}** ±210.29	**32.33^{B}** ±0.41	**1,455.13^{B}** ±32.07	**57.62^{H}** ±1.31	**2.51^{B}** ±0.19	**52.75^{H}** ±2.09	**45.79^{E}** ±1.08
	F	660.88	66.86	66.84	490.93	80.94	1,094.07	73.44
	P <0.05	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

Note: Means in each column followed by same letters are not significantly different at *P* 0.05 by one-way ANOVA with Duncan's multiple range tests.

Table 3. Statistical data of Fe, Cu, Mn, Cr, Cd, Ni, and Pb concentrations (mg kg^{-1}) in leaves of maize plants (*Zea mays* L.) grown in soil samples from polluted locations in Drenas, Kosovo (L1-L9) and a non-polluted location of Budakovë, Suharekë, Kosovo (control).

		Leaves samples – heavy metals						
		Fe	Cu	Mn	Cr	Cd	Ni	Pb
Non-polluted region	**Control group**	**57.59^{A-C}** ±11.61	**2.78^{C}** ±0.11	**95.41^{A}** ±2.67	**0.23^{CD}** ±0.11	ND	ND	ND
Polluted regions	**L1**	**72.45^{A}** ±12.15	**5.89^{A}** ±0.19	**89.97^{A}** ±1.20	**0.68^{B-D}** ±0.05	ND	1.97^{A} ±0.40	ND
	L2	**62.14^{AB}** ±6.79	**4.55^{AB}** ±0.18	**52.65^{DE}** ±2.71	**1.73^{A}** ±0.72	ND	0.71^{B} ±0.62	ND
	L3	**60.50^{A-C}** ±11.79	**5.01^{AB}** ±1.08	**65.38^{C}** ±2.94	**0.52^{B-D}** ±0.03	ND	ND	ND
	L4	**35.87^{BC}** ±3.71	**4.25^{A-C}** ±0.21	**68.25^{C}** ±1.06	**0.52^{B-D}** ±0.23	ND	ND	ND
	L5	**49.98^{A-C}** ±2.73	**5.88^{A}** ±1.12	**50.12^{E}** ±1.48	**0.69^{B-D}** ±0.10	ND	ND	ND
	L6	**48.48^{A-C}** ±7.76	**4.34^{A-C}** ±0.14	**78.22^{B}** ±2.27	**0.61^{B-D}** ±0.23	ND	ND	ND
	L7	**43.64^{A-C}** ±10.16	**3.66^{BC}** ±0.01	**58.38^{D}** ±2.02	**1.39^{AB}** ±0.51	ND	ND	ND
	L8	**52.52^{A-C}** ±9.70	**4.20^{A-C}** ±0.26	**66.41^{C}** ±2.80	**1.02^{A-C}** ±0.20	ND	ND	ND
	L9	**27.87^{C}** ±16.49	**4.25^{A-C}** ±0.17	**76.29^{B}** ±0.69	**0.03^{D}** ±0.03	ND	ND	ND
	F	1.68	3.31	49.27	3.50	.	7.44	.
	P<0.05	**0.159**	**0.112**	**0.001**	**0.009**	.	**0.001**	.

Notes: Means in each column followed by same letters are not significantly different at *P* 0.05 by one-way ANOVA with Duncan's multiple range tests. ND – under limit of detection cy ICP-OES.

concentration of 0.23 mg kg^{-1}. Our study clearly shows that metal concentrations in plants are closely related to metal concentrations in soil. Similar results on metal accumulation in the relationship between soil and maize have been reported by different authors.[45–47]

Biochemical and molecular analyses

The results for the activity of ALA-D, ALA content, GSH levels, MDA levels, TAC levels, and total chlorophyll content are presented in Table 4. The activity of ALA-D was measured in the leaves of maize plants from different locations, taking into account the formation of porphobilinogen. The results show that the enzyme ALA-D had the lowest activity at locations one-L1 (4.45 µmol PGB mg protein^{-1} h^{-1}) and two-L2 (4.52 µmol PGB mg protein^{-1} h^{-1}) compared to the control location (5.74 µmol PGB mg protein^{-1} h^{-1}). The same trend is observed in chlorophyll content, which had lower values in location one-L1 (1.89 mg g^{-1}) compared to the control (2.16 mg g^{-1} DW). Our study shows that soil pollution with heavy metals, especially Ni and Cr, affects the activity of ALA–D and chlorophyll content. Inhibition of the activity of ALA-D leads to

Table 4. Activity of –aminolevulinic acid dehydratase (ALA-D), aminolevulinic acid (ALA), reduced glutathione (GSH), malondialdehyde (MDA), total antioxidant capacity (TAC) and total chlorophyll content (Chl).

		ALA-D (μM PGB mg prot.$^{-1}$ h^{-1})	ALA (μM g^{-1} FW)	GSH (μM g^{-1} FW)	MDA (μM g^{-1} FW)	TAC (μM mg^{-1} FW)	Chl (mg g^{-1} FW)
Non-polluted region	**Control group**	**5.74^{A}** ±0.59	**22.29^{B}** ±2.71	**1.91^{D}** ±0.04	**2.72^{CD}** ±0.07	**1,139.49^{BC}** ±0.59	**2.16^{A}** ±0.05
Polluted regions	**L1**	**4.45^{B}** ±0.25	**33.97^{AB}** ±5.85	**2.62^{AB}** ±0.04	**3.12^{BC}** ±0.14	**1,406.00^{AB}** ±98.88	**1.89^{B}** ±0.06
	L2	**4.52^{B}** ±0.38	**28.19^{AB}** ±3.16	**2.83^{A}** ±0.11	**3.39^{AB}** ±0.10	**1,080.48^{C}** ±21.52	**2.05^{AB}** ±0.04
	L3	**5.04^{AB}** ±0.45	**31.81^{AB}** ±4.71	**2.57^{AB}** ±0.07	**3.88^{A}** ±0.26	**1,484.50^{A}** ±87.85	**2.07^{AB}** ±0.03
	L4	**5.41^{AB}** ±0.25	**34.36^{AB}** ±0.98	**2.44^{A-C}** ±0.24	**3.86^{A}** ±0.14	**1,140.89^{BC}** ±173.54	**2.10^{AB}** ±0.13
	L5	**4.96^{AB}** ±0.40	**37.75^{A}** ±4.08	**2.01^{CD}** ±0.22	**3.01^{B-D}** ±0.08	**1,607.44^{A}** ±158.19	**2.01^{AB}** ±0.03
	L6	**5.69^{A}** ±0.22	**33.07^{AB}** ±2.43	**2.20^{B-D}** ±0.25	**2.48^{D}** ±0.25	**1,433.16^{AB}** ±115.77	**2.11^{AB}** ±0.05
	L7	**5.29^{AB}** ±0.31	**34.92^{A}** ±2.36	**2.43^{A-C}** ±0.16	**3.31^{A-C}** ±0.16	**1,025.70^{C}** ±18.32	**2.14^{A}** ±0.06
	L8	**5.69^{A}** ±0.39	**34.24^{AB}** ±6.19	**2.29^{B-D}** ±0.16	**3.52^{AB}** ±0.38	**1,425.32^{AB}** ±75.53	**1.97^{AB}** ±0.10
	L9	**5.68^{A}** ±0.22	**36.69^{A}** ±0.85	**2.03^{CD}** ±0.04	**2.92^{B-D}** ±0.18	**1,138.48^{BC}** ±23.86	**2.12^{AB}** ±0.06
	F	1.970	1.429	3.454	5.189	4.166	1.430
	P<0.05	**0.069**	**0.209**	**0.003**	**0.000**	**0.001**	**0.208**

Note: Means in each column followed by same letters are not significantly different at *P* 0.05 by one-way ANOVA with Duncan's multiple range tests.

Table 5. The value of DNA content (pg/2C), G1%, S% and G2M% on leaves of maize plants (*Zea mays* L.) grown in soil samples from some polluted locations of Drenas, Kosovo (L1–L9) and non-polluted location of Budakovë, Suharekë, Kosovo (control).

		pg/2C DNA	G1%	S%	G2M%
Non-polluted region	**Control group**	**5.16^{BC}** ±0.04	**75.43^{A}** ±0.42	**4.00^{A}** ±0.14	**20.56^{B}** ±0.28
Polluted regions	**L1**	**5.15^{BC}** ±0.06	**69.76^{AB}** ±4.52	**5.26^{A}** ±2.06	**24.96^{B}** ±3.09
	L2	**5.23^{BC}** ±0.07	**66.29^{A-C}** ±10.08	**3.05^{A}** ±0.70	**30.65^{AB}** ±10.60
	L3	**5.19^{BC}** ±0.04	**60.58^{A-D}** ±7.06	**3.03^{A}** ±1.80	**36.28^{AB}** ±8.49
	L4	**5.15^{BC}** ±0.01	**63.65^{A-D}** ±8.47	**2.14^{A}** ±0.37	**34.20^{AB}** ±8.10
	L5	**5.39^{AB}** ±0.15	**52.38^{B-D}** ±1.76	**3.32^{A}** ±0.43	**44.28^{A}** ±2.14
	L6	**5.25^{BC}** ±0.02	**50.45^{CD}** ±3.58	**3.46^{A}** ±1.18	**46.08^{A}** ±3.08
	L7	**5.30^{A-C}** ±0.04	**63.88^{A-D}** ±5.49	**4.00^{A}** ±1.07	**32.11^{AB}** ±5.98
	L8	**5.54^{A}** ±0.26	**60.27^{A-D}** ±1.55	**3.98^{A}** ±0.54	**35.73^{AB}** ±1.04
	L9	**5.07^{C}** ±0.12	**46.17^{D}** ±2.66	**4.31^{A}** ±0.98	**49.51^{A}** ±3.59
	F	2.79	2.72	0.59	2.59
	P<0.05	**0.027**	**0.030**	**0.784**	**0.036**

Note: Means in each column followed by same letters are not significantly different at *P* 0.05 by one-way ANOVA with Duncan's multiple range tests.

accumulation of ALA in the leaves of maize plants, as shown in Table 4, resulting in higher levels in the areas around the Ferronikel smelter. For example, at location nine-L9, higher ALA values (36.69 μM g^{-1} FW) are observed compared to the control (22.29 μM g^{-1} FW). Our results of ALA-D enzyme activity clearly showed that this enzyme is very sensitive due to heavy metals pollution even on lower concentration. On other hand, strongly effects of ALA-D activity inhibited was reported from other studies on onion plants cultivated in some industrial areas with higher concentration of lead in Mitrovica, Kosovo.[8] Based on this, our results of ALA-D activity and ALA content are in harmony with other authors. In this case, Scarponi and Perucci[48] provided data on the effect of heavy metals (Mn, Fe, Pb, Cu, Zn, and Sn) on ALA-D activity *in vivo* and *in vitro*, indicating activation or inhibition depending on the metal. Gupta et al.[49] showed that exposure of maize plants to Hg inhibited ALA-D activity, while Sarangthem et al.[50] found that exposure to cadmium inhibited enzyme activity and decreased total chlorophyll content in maize leaves. Ahmed et al.[51] reported that Pb concentration inhibited ALA-D activity and caused a significant decrease in total chlorophyll content in *Rhaphanus sativus* plants. Pereira et al.[52] observed inhibition of ALA-D activity in *Cucumis sativus* plants exposed to Al ions. In a study by Calgaroto et al.[53] on *Pfaffia glomerata*, inhibition of ALA-D activity

Table 6. Correlation between heavy metals in plant leaves, biochemical and molecular parameters.

	ALA-D	ALA	GSH	MDA	TAC	Chl	cDNA	G1	S	G2M
Fe	−0.218	−0.214	0.262	0.024	0.266	−0.105	0.107	**0.402***	0.266	**−0.431***
Cu	−0.244	0.217	0.219	0.162	**0.431****	**−0.321***	0.192	−0.085	0.189	0.053
Mn	0.165	−0.246	−0.202	**−0.305***	−0.038	0.030	−0.314	0.311	0.269	−0.343
Cr	−0.178	−0.061	**0.418****	0.244	−0.085	0.050	0.370	−0.086	−0.016	0.087
Ni	**−0.410****	0.004	**0.365****	−0.020	0.005	**−0.365****	−0.106	0.156	0.151	−0.174
ALA-D										
ALA	0.028									
GSH	−0.180	−0.103								
MDA	0.043	0.070	**0.424****							
TAC	0.119	0.117	−0.116	0.032						
Chl	0.140	−0.157	0.134	0.131	−0.202					
cDNA	0.261	0.187	−0.043	0.217	**0.355***	−0.134				
G1	−0.136	**−0.354***	0.181	0.172	−0.227	−0.138	−0.058			
S	−0.053	0.105	−0.157	−0.215	−0.020	−0.099	−0.101	0.093		
G2M	0.144	**0.331***	−0.168	−0.148	0.219	0.150	0.072	**−0.989****	−0.240	

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

was observed in the presence of 50 μM Zn. Vajpayee et al.[54] reported an increase in ALA content in *Nymphaea alba* plants under the influence of chromium, along with a decrease in protein and total chlorophyll content. Similarly, Cenki et al.[55] observed an increase in ALA content in *Brassica rapa* L. plants after exposure to lead (Pb).

On the other hand, heavy metal exposure in plants leads to an increase in GSH and MDA levels, as well as changes in total antioxidant capacity (TAC). The highest GSH levels were observed in leaves of plants grown in location two-L2 soil (2.83 μM g^{-1} FW) and control (1.91 μM g^{-1} FW). In this case, these results of GSH and MDA increase indicate for higher oxidative stress level in cells maize plant and we presume that result from heavy metals higher concentration in these localities. Similar results reported from other authors, Khan et al.[56] reported an increase in GSH levels with increasing Ni concentration in *Brassica juncea*. Ruiz et al.[57] found increased GSH levels in *Helianthus annuus* plants under the influence of Al ions, and Mostofa et al.[58] observed a similar trend with Cu ions in *Oryza sativa*. As for malondialdehyde (MDA) levels, the highest values were measured at location three-L3 (3.88 μM g^{-1} FW), while the lowest value was measured at location six-L6 (2.48 μM g^{-1} FW), with control plants having an MDA value of 2.72 μM g^{-1} FW. Our results for higher MDA level in plant leaves which growth in localities with higher concentration of heavy metals are in line with other authors. Rizvi and Khan[59] demonstrated an increase in lipid peroxidation levels in maize plants as a function of the concentration of heavy metal ions such as Cd, Ni, and Cr in the substrate. Similar findings of increased MDA levels in maize plants were reported under the influence of Cu[60] and with different concentrations of Pb and Cu in the substrate.[61]

Regarding TAC, our study showed that the highest values were recorded at location three-L3 (1,484.50 μM mg^{-1} FW), while the control had the lowest value (1,139.49 μM mg^{-1} FW) and the seventh location recorded a value of 1,025.70 μM mg^{-1} FW. Márquez-García et al.[62] presented data showing an increase in TAC values with increasing Cd concentration up to a certain point, followed by a decrease in *Erica andevalensis* sp. Gjorgieva et al.[63] found that various heavy metals such as Cu, Mn, Pb, Ni, Zn, and Cd at different concentrations caused changes in TAC levels in *Phaseolus vulgaris* sp. In a study conducted by Kulbat-Warycha et al.[64] on *Origanum vulgare* L., exposure to different concentrations of Ni, Cu, and Zn caused changes in TAC values compared to unexposed plants. In particular, low concentrations of Ni and Cu caused an increase in TAC values, while higher concentrations caused a significant decrease in TAC values.

Pursuant to various studies, the effects of heavy metals on DNA content and cell cycle phases (G1, S, G2M) were investigated. Table 5 shows the data related to the molecular effects of heavy metals on DNA content and cell cycle phases. Our study revealed the highest DNA content in leaf samples from location eight-L8 (5.54 pg/2C), while the lowest values were observed at location nine-L9 (5.07 pg/2C) and in the control (5.16 pg/2C). Numerous studies have demonstrated the relationship between heavy metals and genome size. Monteiro et al.[25] reported that DNA content depends on Cd concentration, plant species, and the specific organ analyzed (e.g., root or leaf). In leaf samples of *Lactuca sativa* and *Thlaspi arvense*, DNA content decreased with increasing Cd concentration, while *Thlaspi caurlescens* showed an increase in DNA content. Abdelhaliem and Al-Shalawi[65] observed a decrease in DNA content with increasing Pb concentration in *Glycine max*. A similar decrease in DNA content was observed in *O. sativa* treated with Al.[66] However, Slomka et al.[27] found no significant changes in DNA content in *Viola tricolor* grown under natural conditions in soil polluted with metals (Zn, Pb, Cu, Cd) compared to non-polluted soil. Differences on DNA content between same species growth in different locations also have reported from Tuna et al.[67]

Regarding the distribution of cells across cell cycle phases, the highest percentage was observed in G1 stage in the control group (G1-75.43%), while the lowest value was recorded in the ninth locality L9 (G1-46.17%). No significant differences were observed in the S phase. In the G2M phase, the highest value was observed in the ninth location L9 (G2M-49.51%), while the control had the lowest value (G2M-20.56%). Many studies have demonstrated the influence of heavy metals on cell cycle dynamics. Cui et al.[68] studied the effect of different Cd concentrations on *Arabidopsis thaliana* and found a decrease in the proportion of cells in G1 phase and an increase in G2M phase with

increasing Cd concentration. Rodriguez et al.[69] conducted a study on *Pisum sativum* under abiotic stress with different doses of Pb and showed similar trends in cell cycle distribution. The effects of different concentrations of Cr (VI) on *P. sativum* in terms of cell cycle phase distribution also showed an increase in the proportion of cells in the G2 phase.[70] Youssef et al.[71] found an increased cell distribution in the G2 phase in *Z. mays* after exposure to Cd.

The correlation between heavy metals in maize leaves plant and various biochemical and molecular parameters are presented in Table 6. The data obtained show a significant negative correlation between ALA-D activity and the content of total chlorophylls with nickel (Ni). This indicate that, nickel can have inhibition effect of ALA-D activity which also reflects in the amount of chlorophylls in the later steps, because it is the primary enzyme in the path of their biosynthesis. Conversely, a significant positive correlation is observed between GSH and nickel (Ni) and chromium (Cr). Copper (Cu) shows a significant positive correlation with TAC. Also, a significant negative correlation is observed between MDA and manganese (Mn). Above all, iron (Fe) shows a strong correlation with cell cycle dynamics, with a significant positive correlation with G1 and a significant negative correlation with G2M.

In biochemical parameters, a significant positive correlation is observed between GSH and MDA, indicating the toxicity of heavy metals indicated by these parameters. Also, a significant positive correlation is observed between cDNA and TAC, while a significant negative correlation is observed between G1 and ALA. In addition, a significant positive correlation is observed between G2M and ALA. In terms of cell cycle dynamics (G1, S, G2), a significant negative correlation is observed between G1 and G2, which is a clear evidence of the influence of heavy metals on cell cycle dynamics.

Conclusions

The concentration of heavy metals in soil samples and leaves of maize plants was higher in the polluted regions around Ferronikeli in Drenas, Kosovo, than in the village of Budakovë in municipality, Suharekë, Kosovo. Furthermore, the higher concentration of heavy metals, especially Ni and Cr with ten or more times higher was conducted in localities vicinity to smelter or at waste collection points of Feronikeli smelter (L2, L3 and L1). These results indicate that agricultural soils which are surrounded to smelter have potentially harmful effects for all living organisms. Some metals (especially Ni and Cr) exceed the permissible metal concentration values in agricultural soils according to the European standards, although the specific elements that exceed the limits are not specified.

Heavy metals, particularly Ni, Fe, Cr and Cu, have a direct influence on various biochemical and molecular parameters. Significant negative correlation between Ni accumulation in the maize leaves and ALA-D activity and chlorophyll content was observed.

In addition, heavy metals affect the amount of nuclear DNA and the phases of the cell cycle (G1, S, G2). Significant differences in DNA content were found between the analyzed groups of maize leaves. Particularly, significant differences are observed in the dynamics of the cell cycle, especially in the G1 and G2 phases, while no significant differences are observed in the S phase. Under the influence of heavy metals, a lower rate of cell distribution in G1 phase and an increased rate of cell distribution in G2 phase are generally observed. This can be considered as a protective strategy to eliminate errors in the genetic material by repair mechanisms or even as a step toward programmed genetic death.

The maize plant, a common crop worldwide, serves as a suitable model for studying the potential toxicity resulting from contamination of agricultural soils with heavy metals. Systematic monitoring of agricultural soils near various sources of heavy metals is necessary to ensure a healthy ecosystem.

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Chlorophyll biosynthesis suppression, oxidative level and cell cycle arrest caused by Ni, Cr and Pb stress in maize exposed to treated soil from the Ferronikel smelter in Drenas, Kosovo

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ABSTRACT

Although certain trace elements are essential for normal plant functionality, an excessive increase in their concentration can disrupt plant development and physiology due to phytotoxicity. This study aims to determine the toxic tolerance limits for different concentrations of Ni and Cr (50, 100, 200, and 400 ppm) and Pb (20, 50, 100, and 200 ppm) in maize seedlings grown in soil collected near the Ferronikel smelter in Drenas, Kosovo. We will assess these limits using sensitive biomarkers, including δ-aminolevulinic acid dehydratase (ALA-D) activity, δ-aminolevulinic acid (ALA) content, chlorophyll content, glutathione (GSH) levels, and lipid peroxidation (MDA), as well as by evaluating DNA content and cell cycle dynamics. All the investigated heavy metals showed a significant increase in concentration in leaves; in particular, Ni showed a strong significant association between its concentration in treatment and in the leaves. At concentrations of 400 ppm, Ni and Cr had significant negative effects on all biomarkers, with ALA-D activity inhibited by up to 50%, and total chlorophyll content significantly decreased. A robust correlation was observed between Ni and Cr and the level of cellular oxidative stress in leaves, as monitored through GSH, lipid peroxidation, and ALA levels. Additionally, the cell cycle, especially in the G1 and G2/M phases, was arrested. These findings emphasize the significant adverse impact of high concentrations of Ni and Cr in plant metabolism. This research contributes to our understanding of managing and mitigating heavy metal contamination in agricultural areas and its potential implications for plant defense mechanisms.

1. Introduction

Heavy metal pollution is a consequence of anthropogenic activities, notably urbanization and industrialization, and it exerts adverse effects on plant physiology and development through phytotoxicity (Khan et al., 2015). The concentrations of heavy metals in soil have seen a substantial increase due to escalating anthropogenic environmental pollution from various sources, including metallurgical processes (such as mining and foundry operations), agriculture (involving the use of fertilizers and pesticides), energy production, fuel combustion, microelectronic manufacturing, and industrial waste disposal. Among these stressors, one of the most significant factors adversely affecting plant growth and productivity is the contamination of soil by heavy metals (Mansoor et al., 2023; Fryzova et al., 2018; Kumar et al., 2016).

Heavy metals include specific metals and metalloids characterized by an atomic number greater than 20 and/or a density greater than 5 g/cm^3. They are well known for their persistence, nonbiodegradability, high toxicity, and easy transmission through the food chain. These metals exert cytotoxic, genotoxic, and mutagenic effects on humans, animals, and plants, contaminating various components of ecosystems, including food chains, soil, irrigation or drinking water sources, aquifers, and the surrounding atmosphere (Flora et al., 2008; Kim et al., 2015; Ali et al., 2019). Based on their biological significance, heavy metals can be categorized into two groups: essential micronutrients vital for normal plant growth (such as Fe, Mg, Mn, Mo, Cu, Zn, and Ni) and nonessential elements lacking known physiological or biological

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functions (including Pb, Cd, Co, Cr, As, Ag, Se, Sb, and Hg) (Kabir et al., 2012; Emamverdian et al., 2015; Mitra et al., 2022). The essential micronutrients play a critical role in the structure of proteins and enzymes, which are vital for normal plant metabolism, growth, and development. Plants need a small amount of these metals, but both essential (such as Ni, Cu and Zn) and non-essential (such as Pb, Cd and Cr) metals in high concentrations can lead to the reduction or inhibition of growth in plants (Zengin and Munzuroglu, 2005; Ali et al., 2019). In line with this, heavy metals at elevated or toxic concentrations can disrupt normal physiological processes in plants through various mechanisms. They can form bonds with sulfhydryl groups, leading to structural changes in protein building blocks (Hall, 2002), displace or interfere with the functioning of essential metals in biomolecules like enzymes, cytochromes, or pigments (Ali et al., 2013), and adversely affect cytoplasmic membrane integrity (Mansoor et al., 2023). Consequently, these disruptions suppress crucial plant processes, including photosynthesis, respiration, and enzyme activity (Fryzova et al., 2018; Gashi et al., 2020). Elevated concentrations of heavy metals are associated with increased production of reactive oxygen species (ROS). In such conditions, ROS can harm plants in various ways, including protein oxidation, enzyme inhibition, lipid peroxidation, and damage to RNA and DNA. Additionally, ROS play a pivotal role in regulating gene expression under plant stress conditions, affecting the cell cycle, and ultimately leading to programmed cell death (Mansoor et al., 2023).

Furthermore, it is widely recognized and traditionally established that one of the most detrimental impacts of heavy metals stems from the inactivation of enzymes and biomolecules due to the displacement of essential metal ions from specific binding sites (Juknys et al., 2012; Ali et al., 2019). These adverse conditions in the plant cells, induced by heavy metals, also disrupt the chlorophyll synthesis pathway, particularly the enzymatic processes involved in the initial stages, from porphobilinogen synthesis to protoporphyrin IX formation (Jaffe, 2000; Rai et al., 2016; Gashi et al., 2020).

Chlorophyll synthesis stands as a pivotal and indispensable process in plant cells, representing a fundamental biomolecule crucial for plant physiology and development, even under stressful conditions. The initiation of the chlorophyll synthesis pathway, as well as the production of other tetrapyrrole derivatives in plant cells, is catalyzed by δ-aminolevulinic acid dehydratase (ALA-D). This enzyme condenses two molecules of δ-aminolevulinic acid (ALA) to produce porphobilinogen (Jaffe, 2000). ALA-D in plant cells operates as a metalloenzyme, and its activity is regulated by magnesium (Mg) or it can utilize various forms of divalent or monovalent cations (Jaffe, 2000). However, when high concentrations of divalent heavy metal ions are present, they inhibit the activity of ALA-D, resulting in an accumulation of ALA (Gupta et al., 2013). Notably, ALA-D enzyme has gained recognition as a highly sensitive biomarker for detecting heavy metal pollution in the environment and is often considered the premier choice for environmental biomonitoring (Gashi et al., 2020; Buqaj et al., 2023; La-Llave-León et al., 2017; Tian et al., 2013; Bollinger et al., 2023). The primary harmful effects of heavy metals at higher concentrations have been suggested to include the inhibition of ALA-D activity, under both '*in vivo*' and '*in vitro*' conditions (Gupta et al., 2013). Another deleterious cellular consequence is the accumulation of ALA, which promotes the generation of reactive oxygen species (ROS), leading to an increase in cellular oxidative stress (Osmani et al., 2018). In summary, the negative impact of heavy metals is generally associated with the inhibition of ALA-D activity on one hand and an increase in ALA levels on the other hand.

Furthermore, besides the generation of reactive oxygen species (ROS), heavy metals within plant cells disrupt antioxidative defense mechanisms, primarily by affecting glutathione and binding to sulfhydryl groups on antioxidative enzymes (Hasanuzzaman et al., 2017; Rizvi et al., 2019). These harmful conditions induced by heavy metals elevate oxidative stress levels, leading to cellular damage and disrupting cellular ionic balance. Specifically, glutathione (GSH) and other thiol compounds play a crucial role in chelation mechanisms, maintaining lower metal ion concentrations and detoxifying free metal ions, primarily within the plant cell's cytoplasm (Seth et al., 2012). GSH, a tripeptide (γ-Glu-Cys-Gly), is widely distributed in intracellular compartments and serves as an antioxidant pivotal to plant defense mechanisms. It also acts as a precursor for the synthesis of phytochelatins, a family of peptides structurally related to GSH, in metal-exposed plants (Anjum et al., 2015). It is worth noting that the system of cell membranes, particularly the membranes of chloroplasts, is frequently identified as the primary target of oxidative effects induced by heavy metals. The primary constituents of membrane lipids, polyunsaturated fatty acids, are highly susceptible to oxidation. Malondialdehyde (MDA), a natural byproduct of lipid peroxidation, serves as a critical indicator of the health of cellular lipids, particularly those involved in cell membrane formation (Valko et al., 2005). Additionally, the quantification of MDA in plants or plant cells serves as an indicator of overall cellular condition and the level of oxidative stress

There are a few of studies that have explored the effects of heavy metals on DNA nuclear content and cell cycle dynamics, including G1, S, and G2. Flow cytometry (FCM) offers a valuable approach, combining rapid analytical capabilities with multiparametric analysis, encompassing DNA content, clastogenicity, ploidy changes, and cell cycle dynamics. Previous research has successfully used FCM to detect genotoxicity resulting from exposure to chromium and mercury in peas (Azevedo et al., 2005; Cao et al., 2018; Monteiro et al., 2010).

In recent years, several studies have been conducted on soil contamination by heavy metals in various industrial areas in Kosovo resulting from smelting or mining activities, both in Mitrovica and Drenas. However, detailed investigations regarding the effects on living organisms and the extent of harmful toxicity remain limited (Borgna et al., 2008; Nannoni et al., 2011; Zogaj et al., 2016; Gashi et al., 2020). These studies have presented findings on heavy metal concentrations (Cd, Cr, Cu, Ni, Pb, and Zn) in soils, as well as their potential transfer and bioaccumulation factors in different plant species. Furthermore, in our prior study, significantly higher concentrations of Ni and Cr were discovered in agricultural soils near the Ferronikeli smelter in Drenas, Kosovo (Buqaj et al., 2023). In this polluted area we founded 809 mg kg^{-1} for nickel (Ni), 801 mg kg^{-1} for chromium (Cr), 41109 mg kg^{-1} for iron (Fe), and 56 mg kg^{-1} for lead (Pb). In the non-polluted region, the concentration of these metals was 20 mg kg^{-1} for Ni, 28 mg kg^{-1} for Cr, 19166 mg kg^{-1} for Fe, and 44 mg kg^{-1} for Pb (Buqaj et al., 2023). Based on these results, in this study we used solutions with a range of metal concentrations commonly encountered in the area of Drenas, Kosovo, from lower to higher concentrations than measured in this soil. The metal concentrations used in this experiment corresponded to the concentrations of these metals, especially Ni and Cr, within their levels of toxicity that inhibited plant physiology and development. Therefore, the main purpose of this study is to quantify the maximum toxic thresholds for Ni, Cr and Pb when applied to soil from the area in the vicinity of the Ferronikeli smelter in Drenas. Additionally, we aim to forecast potential harmful effects on plants resulting from various concentrations of these metals, thus determining their toxic tolerance limits for future reference. To assess the extent of toxic effects and oxidative stress induced by these heavy metals on maize plants, we used sensitive biomarkers, including δ-aminolevulinic acid dehydratase (ALA-D) activity, δ-aminolevulinic acid (ALA) content, chlorophyll content, glutathione (GSH) levels, lipid peroxidation (MDA), as well as evaluating DNA content and cell cycle dynamics. To achieve this objective, maize seedlings were grown in soil collected near the Ferronikel smelter in Drenas in Kosovo, and treated with salt solutions of different concentrations of Ni and Cr (50, 100, 200, and 400 ppm) and Pb (20, 50, 100, and 200 ppm). Notably, this study represents the first comprehensive evaluation of the impact of these heavy metals when introduced into contaminated soil within an industrial zone.

2. Material and methods

2.1. Soil sampling and plants exposed to heavy metals

Soil samples were collected with a random procedure in agricultural areas from a polluted region in the vicinity of Ferronikeli Smelter in Drenas, Kosovo, and from a non-polluted region (control site) in Budakovë village, Suharekë municipality, Kosovo. Test samples were taken at a distance from 500 to 1000 m from the smelter, and one sample represents agricultural soils taken from different points with a radius of about 50 m at different poles. After the soil dried, all collected samples from each region were mixed well and divided into two groups. The first group of soil samples was used for maize seedling growth without any treatment and the second group for maize seedling growth with soil treated by various heavy metal (Ni, Cr and Pb) concentrations. According to our recent study of heavy metal concentrations in these soil samples, the level of Ni and Cr were higher in concentration compared to the control site (Buqaj et al., 2023). Based on these results, we used solutions with a range of metal concentrations (Ni, Cr and Pb) encountered in this polluted area, from lower to higher concentrations than in this soil. The concentrations used for heavy metals were as follows: 50, 100, 200 and 400 ppm of Ni and Cr, 20, 50, 100 and 200 ppm of Pb. These concentrations of heavy metals corresponded also with other studies used for *in vitro* toxicity testing and their harmful effects on different plant species (Juknys et al., 2012; Georgiadou et al., 2018; El-Shora et al., 2021). Taking into account that metals differ in their toxicity, a particular range of investigated concentrations was chosen for each heavy metal used. The great importance of the selection of these metal concentrations serves a dual purpose: to determine the toxic dose limits and to evaluate potential risk assessments for the future, should metal concentrations continue to increase.

2.1.1. Plant cultivation and heavy metal treatments

For each treatment of heavy metals, untreated soil and control, a suitable quantity of dry soil was utilized, combined with perlite in a 3:1 ratio (the final amount for each treatment was 2 kg per pot). Subsequently, the soil mixture from group 1 was subjected to treatment with specific salt solutions corresponding to each heavy metal treatment, including ($NiCl_2 \cdot 6H_2O$, $CrCl_3 \cdot 6H_2O$ and $PbCl_2$). This treatment aimed to achieve the desired metal concentrations within the soil, specifically 50, 100, 200, and 400 ppm for Ni and Cr, and 20, 50, 100, and 200 ppm for Pb. Following the addition of these solutions, the contaminated soils were allowed to sit for a period of five days; this duration allowed for the binding of heavy metals with the soil matrix. Subsequently, the soils were adequately hydrated for further experimentation.

The maize seeds were sterilized with 70% ethanol for 2 min, rewashed with distilled and deionized water, and germinated on moist filter paper in Petri dishes. Germinated seedlings were carefully transferred into individual vegetation vessels, each containing treated soil corresponding to the designated heavy metal treatment. Furthermore, the maize seedlings were subjected to stress according to the respective treatment from the first day of cultivation. Three replicates were maintained for each heavy metal treatment. The maize seedlings were cultivated under controlled greenhouse conditions for approximately four weeks. Between the 21st and 25th days after maize planting, the first and second leaves of each seedling were selected for subsequent analyses. The harvested leaves were categorized into three distinct types for the following analysis: the first samples of leaves was dried and used for heavy metal analyses; the second samples of leaves was utilized for DNA content and cell cycle analysis; and the third samples of leaves was reserved for physiological and biochemical analyses.

2.1.2. Heavy metal analyses

The analysis of heavy metals was carried out to determine their concentrations in leaves of the maize plants, both for those metals that were added to the soil, as well as for some other metals such as: Cd, Cu, Fe and Mn. The maize leaves immediately after harvesting were rinsed with deionized water, dried at 105°C for about 24 h and ground to a fine powder. For digestion of plant samples, we used a microwave digester (Berghof). Leaf samples were solubilized by acid digestion in Teflon bombs by adding 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 to about 500 mg of powered sample. To determine heavy metal concentrations in the leaf samples, an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was utilized. The analysis was conducted at the analytical laboratory Agrovet located in Fushë Kosovë, Kosovo.

2.2. Analysis of the chlorophyll biosynthesis pathway

2.2.1. Assay of enzyme δ-aminolevulinic acid dehydratase (ALA-D) activity

The enzyme was determined colorimetrically using a modified Ehrlich reagent to determine the amount of PBG (porphobilinogen) formed. The extraction and assay of ALA-D were conducted following the procedure outlined by Jain and Gadre (2004), slightly modified by Osmani et al. (2018) The enzyme was extracted using ice-cold 50 mM Tris-HCl buffer (pH 8.4) containing 0.2% Triton X-100. Leaves of similar size were obtained from ten plants, and each 500 mg of leaf tissue was ground into a fine powder in an ice-cold mortar. To prevent phenol oxidation, 1 g of polyvinylpolypyrrolidone was immediately mixed with the powdered tissue after grinding. After filtration, the homogenate was centrifuged at 15,000 g for 20 min at 4°C. The pellet was discarded, and the supernatant served as the enzyme source. To initiate the reaction, 1 mL of the enzyme was incubated with 0.27 mL of 1 mg/mL ALA, 1.35 mL of 50 mM Tris-HCl buffer (pH 8.5), and 0.08 mL of 0.02 M $MgCl_2$. The reaction proceeded for 1 h with gentle shaking (150 rpm) at 37°C. The reaction stopped was stopped by adding 0.3 mL of 3 M TCA, followed by centrifugation at 5000 g for 10 min. PBG estimation involved mixing the supernatant with Ehrlich reagent (prepared fresh by dissolving 1 g of 4-dimethyl aminobenzaldehyde in 30 mL of glacial acetic acid and 8 mL of 70% PCA, then adjusting the volume to 50 mL with glacial acetic acid) at a 1:1 (v/v) ratio. The absorbance was measured at 553 nm after 15 min and compared to a zero-time control. Enzyme activity was defined as 1 nmol of PBG formed per hour, and protein content was determined following the Bradford method (Bradford, 1976).

2.2.2. Determination of δ-aminolevulinic acid (ALA) content

This was determined according to the method of Tewari and Tripathy (1998). Leaves of similar size were collected from ten plants and homogenized using 1 mL of 1 M Na-acetate buffer (pH 4.6). Following homogenization, the samples were subjected to centrifugation at 15,000 x g for 15 min at 4°C. The supernatant obtained from the centrifugation was used for the condensation of δ-aminolevulinic acid (ALA) to produce PBG. This condensation was achieved by mixing 0.7 mL of the supernatant with 0.8 mL of distilled water and 0.1 mL of an ethyl acetoacetate mixture, followed by incubation in a boiling water bath for 10 min (Osmani et al., 2018). After cooling, an equal volume of Ehrlich reagent was added to the mixture. The formation of a colored complex in the solution was measured by absorbance at 553 nm. The amount of PBG formed was quantified using a standard curve generated from ALA, and the results were expressed in μM ALA g^{-1} leaf fresh weight (FW).

2.2.3. Total chlorophyll determination

To extract chlorophyll, 100 mg of fresh maize leaves were utilized, and extraction was carried out using 80% acetone. The chlorophyll content was quantified by measuring absorbance values at 663 nm and 645 nm using a UV–Vis spectrophotometer. In this analysis, updated extinction coefficients and equations from Lichtenthaler (Porra et al., 1998) were applied. The total chlorophyll content was expressed as mg g^{-1} leaf dry weight (DW), providing a quantitative measure of chlorophyll concentration in the plant leaves.

2.3. Oxidative stress level analyses

2.3.1. Determination of glutathione (GSH) content

Fresh leaves from the maize plant were homogenized using a 5% of trichloroacetic acid (TCA) solution. The homogenate was subjected to centrifugation at 15,000 x g for 20 min, and the pH of the resulting supernatant was adjusted to a range between 4.0 and 5.0 by adding 1 M NaOH. The content of GSH in the crude extract was determined using the Ellman method (Primiano and Novak, 1992) which involves the use of DTNB (5,5-dithiobis (2-nitrobenzoic acid)). In this assay, a reaction mixture was prepared by combining 0.1 mL of the sample, 2 mL of 100 mM pH 8.4 Tris–HCl buffer, and 0.1 mL of the Ellman reagent (60 mg/100 mL in Tris–HCl buffer, 0.1 M, pH 7.0). The absorbance of the reaction mixture was read at 412 nm. The concentration of glutathione in the samples was determined by comparing the absorbance values to a standard curve for GSH. The data were expressed as $\mu M\ g^{-1}$ fresh weight of leaf, providing a quantitative measure of GSH concentration in the leaf samples.

2.3.2. Determination of malondialdehyde (MDA) content

To quantify lipid peroxidation, the content of malondialdehyde (MDA) in maize leaves was determined following the method described by Hodges et al. (1999). Fresh leaves (500 mg) from the maize plants were ground in 5 mL of 0.1% trichloroacetic acid (TCA). The resulting homogenate was then subjected to centrifugation at 15,000 x g for 20 min at 4°C. MDA content was assessed utilizing the corrected thiobarbituric acid (TBA) method. In this procedure, 2 mL of the extraction solution was mixed with 3 mL of 0.5% TBA dissolved in 10% TCA, followed by vigorous mixing. The mixture was heated at 95°C in a constant temperature water bath for 30 min and subsequently cooled on ice to reach room temperature. Afterward, the supernatant was obtained by centrifugation at 5000 x g for 10 min, and its absorbance was measured at three different wavelengths, 450 nm, 532 nm, and 600 nm, using a UV–vis spectrophotometer. The MDA concentration was calculated using the following formula: CMDA ($\mu mol\ mL^{-1}$) = 6.45 x (D532 – D600) - 0.56 x D450, where D450, D532, and D600 represent the absorbance values at 450 nm, 532 nm, and 600 nm, respectively. The data were expressed as $\mu M\ g^{-1}$ fresh weight of leaf, providing a quantitative measure of MDA concentration in the leaf samples.

2.4. Molecular analyses

2.4.1. Nuclear DNA content and cell cycle determination

The analysis of nuclear DNA content in maize leaves was conducted following the protocols outlined by Tuna et al. (2020). Suspensions containing intact nuclei were prepared using commercial kits sourced from Partec (Munster, Germany). Fresh leaf tissue from each sample (20 mg) and a standard leaf tissue (*Secale cereale*, 20 mg) were simultaneously minced in a Petri dish containing 0.5 ml of extraction buffer. The homogenized solution was transferred through a 30 μm filter into a glass tube, and then 2 ml of staining buffer (CyStain PI absolute P) was added to each tube.

Before analysis, the samples were incubated at room temperature in the dark for a minimum of 30 min. Fluorescence intensities emitted by the stained nuclei were measured using a Partec CyFlow Space flow cytometer (Munster, Germany), and the acquired data were analyzed using FloMax analysis software specifically designed for this cytometer. The 2C nuclear DNA content of the samples was calculated based on the relative positions of the G1 peaks of the sample and the standard. The G1, S, and G2/M phases of the cell cycle interphase were determined using the flow cytometer following the same procedure outlined above. It is important to note that only maize plant leaves were homogenized for this purpose, while the leaves of the standard plant were excluded from the analysis.

2.5. Data analysis

The experiment was conducted following a randomized design with three replicates. Statistical analyses were carried out using the SPSS 17 statistical software program. Variations between treatments and among parameters were evaluated through one-way ANOVA. Mean comparisons were executed using the Duncan Multiple Range Test at a significance level of 5%.

3. Results and discussion

3.1. Accumulation of heavy metals

The concentrations of heavy metals (Ni, Cr, Pb, Cd, Cu, Fe, and Mn) in maize seedling leaves are presented in Table 1. The concentration of cadmium (Cd) was under the limit of detection in all leaf samples analyzed with ICP-OES, while lead (Pb) was only detected in maize seedling samples treated with higher concentrations of Pb (50, 100, and 200 ppm). Based on these results, it is clear that lead, even though applied at relatively high concentrations in the treatment of maize seedlings, was absorbed in relatively small quantities, and perhaps this can be linked to its very low quantity in the contaminated soil and low absorption by maize. This crucial mechanism of lead tolerance is further confirmed by other studies. Both apoplastic and symplastic transport of lead are significantly restricted in roots, and the Casparian strip, formed in the endodermal cells, plays a significant role in minimizing lead concentration during its transportation within the plant (Verma and Dubey, 2003). This mechanism helps to prevent excessive lead accumulation and potential toxicity within the plant (Collin et al., 2022). The absorption and transport of lead from soil to roots and leaves vary among different plant species. Once absorbed, lead tends to accumulate in the roots, with only a minor portion being transported to the above-ground parts of the plant (Kumar et al., 2017). Consequently, root vegetables such as carrots and sweet potatoes may exhibit the highest levels of lead accumulation (Kumar et al., 2017). Leafy greens like lettuce and Swiss chard follow in terms of lead uptake. Tomatoes, on the other hand, are relatively less prone to absorbing lead from the soil (Aponte et al., 2020).

Regarding nickel (Ni) in leaves of maize seedlings (Table 1), it has been found that there is a strong significant association between Ni concentration in treatment and its concentration in the leaves; Ni concentration in maize seedling leaves reached up to 392.52 mg kg^{-1} in the 400 ppm Ni treatment. Furthermore, Georgiadou et al. (2018) reported that soil uptake of nickel by plants is influenced by both the type and degree of soil contamination, and when the soil contains varying levels of Ni contamination, plants will absorb Ni in significant amounts until a point of toxicity is reached. At this stage, the plant's ability to continue absorbing Ni is compromised due to the adverse effects of excessive Ni uptake. According to our previous study, a higher concentration of heavy metals in soil, especially nickel and chromium (809 mg kg^{-1} and 801 mg kg^{-1}, respectively), with concentrations exceeding ten times the acceptable limits, was observed in this area near the smelter or at waste collection points of the Feronikeli smelter in Drenas, Kosovo (Buqaj et al., 2023). According to our previous study, physicochemical properties of the soil were as following: the pH(H_2O)=7.15, soil organic carbon (C) 4.74%, the total nitrogen (N) 0.26%, the soil texture (Sand 18.59%, Silt 40.76% and Clay 40.65%), and texture class was Silty clay (SC) (Buqaj et al., 2023). These findings strongly suggest that agricultural soils in close proximity to the smelter pose potentially harmful effects on all living organisms. Generally, data from previous studies have consistently shown elevated levels of heavy metals in industrial areas, surpassing permissible standards. For instance, Zogaj & During (2016) reported concentrations of Ni at 258.54 mg kg^{-1} and Cr at 203.22 mg kg^{-1} in agricultural areas near the Ferronikel smelter. Several studies conducted by various researchers in Kosovo (Borgna et al., 2008; Nannoni et al., 2011) have consistently demonstrated an

Table 1
Heavy metal concentrations (Ni, Cr, Pb, Cd, Cu, Fe and Mn) in leaves of maize seedlings (mg kg^{-1}) grown in soil samples: from non-polluted location (control group) and polluted location (untreated soil and soil treated by salts of heavy metals Ni, Cr and Pb in different concentrations).

		Ni	Cr	Pb	Cd	Cu	Fe	Mn
Control group		ND	1.06^{B} ±0.58	ND	ND	4.91^{AB} ±0.40	27.72^{B} ±14.59	32.11^{B} ±2.03
	Untreated soil	0.71^{F} ±0.62	1.73^{C} ±0.72	ND	ND	4.55^{B} ±0.18	62.14^{B} ±6.79	52.65^{I} ±2.71
Polluted soil	Ni 50 ppm	10.45^{D} ±0.33	4.52^{B} ±0.61	ND	ND	3.76^{BC} ±0.27	73.82^{B} ±4.74	84.29^{H} ±1.69
	Ni 100 ppm	24.51^{C} ±2.69	8.02^{B} ±2.37	ND	ND	2.13^{CD} ±0.24	74.02^{B} ±48.45	92.30^{GH} ±5.46
	Ni 200 ppm	130.71^{B} ±2.35	7.92^{B} ±1.46	ND	ND	3.21^{BC} ±0.66	258.19^{B} ±90.38	138.56^{E} ±7.68
	Ni 400 ppm	392.52^{A} ±3.92	7.73^{B} ±0.75	ND	ND	1.18^{D} ±0.09	262.55^{B} ±69.32	167.62^{D} ±3.75
	Cr 50 ppm	6.69^{DE} ±2.31	10.55^{B} ±2.22	ND	ND	3.37^{BC} ±0.22	57.63^{B} ±3.52	106.82^{FG} ±6.68
	Cr 100 ppm	0.99^{EF} ±0.29	9.90^{B} ±0.36	ND	ND	3.84^{BC} ±0.07	57.70^{B} ±6.45	107.38^{FG} ±2.61
	Cr 200 ppm	6.02^{D-F} ±0.36	13.92^{B} ±0.63	ND	ND	3.70^{BC} ±0.48	77.34^{B} ±1.06	258.13^{C} ±1.37
	Cr 400 ppm	22.56^{C} ±2.94	55.25^{A} ±8.07	ND	ND	3.14^{BC} ±0.17	176.34^{A} ±15.22	509.40^{A} ±19.80
	Pb 20 ppm	0.96^{EF} ±0.81	10.08^{B} ±2.98	ND	ND	4.40^{B} ±0.43	64.65^{B} ±39.55	124.40^{EF} ±5.81
	Pb 50 ppm	0.61^{F} ±0.30	6.51^{B} ±1.18	0.06^{C} ±0.06	ND	6.36^{A} ±1.70	37.21^{B} ±13.32	109.19^{FG} ±5.93
	Pb 100 ppm	2.85^{EF} ±0.42	5.93^{B} ±0.24	1.07^{B} ±0.24	ND	4.33^{B} ±0.10	39.03^{B} ±9.05	120.01^{EF} ±0.50
	Pb 200 ppm	2.66^{EF} ±0.45	10.23^{B} ±1.00	2.11^{A} ±0.29	ND	3.11^{BC} ±0.12	61.68^{B} ±1.82	181.25^{D} ±0.44
	F	3590.62	21.51	48.73	.	4.98	7.37	282.58
	$p<0.05$	0.000	0.000	0.000	.	0.000	0.000	0.000

Note. Soil from non-polluted location (control group) in Budakovë village, Suharekë municipality, Kosovo and polluted soil from the vicinity of Ferronikeli Smelter in Drenas, Kosovo. Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

increase in the concentration of these elements in the vicinity of industrial areas, posing a significant environmental risk.

The absorption of chromium (Cr) by maize seedlings was lower compared to nickel (Ni), but the trend remained consistent; the content increased with the rising concentration applied in the soil. The highest amounts were found in the treatment with 400 ppm of Cr, where the concentration in the maize seedlings leaves was 55.25 mg kg^{-1} (Table 1). The Ni concertation significantly increased even after treating the maize seedlings with other metals, such as chromium (Cr) and lead (Pb), compared to the samples from the control or untreated soil. This was especially notable in the treatment with 400 ppm of Cr, where the Ni concentration in leaves was 22.56 mg kg^{-1}. Regarding this increase in the mobility or transfer of metals with the application of the treatment, we have also observed a significant increase for Cr, Fe, and manganese (Mn). In this case, a very strong and significant correlation was observed between the Fe concentration in leaves and the increasing concentrations of Ni treatment, where the Fe content reached up to 262 mg kg^{-1} in the treatment with 400 ppm of Ni. Similar to results with other plant species, nickel can enhance the uptake and transport of iron in plants and influence the expression and activity of transport proteins responsible for iron uptake from the soil into plant roots and shoots (Emamverdian et al., 2015; Khan and Khan, 2010). Another study conducted by Kacálková et al. (2014) focused on different plant species (maize, sunflower, willow, poplar) grown in soils containing varying levels of metals such as Pb, Cr, Cd, Ni, etc. The findings reveal a recurring pattern of metal accumulation not only in the leaves, but also in other parts of the plants. The extent of accumulation is influenced by the specific type of metal and plant, further emphasizing the selective molecular mechanisms at play. Similar results reported by Khair et al. (2020), Mentha piperita seedlings were also treated with different concentrations of nickel (100, 250 and 500 μM). Based on the data, an increase in nickel concentration in the plant leaves was found depending on the increase in the applied dose. According to the data obtained by Mao et al. (2018) in *Glycine max* and *Vigna radiata* plants after their treatment with different doses of metals such as: Pb, Cr, Hg, Cd and Cu, changes in various biochemical parameters were studied - both physiological and in terms of accumulation of metals in different parts of the plants. The concentration of the elements in question in the leaves of the plants depended on the concentration dose, the type of metal and the high-altitude plant species.

In contrast to results of Ni, the concentration of Mn in maize seedlings showed a very strong association with the concentrations of C and, in this study, its content increased significantly with the increase in Cr concentration, as well as with other metals (Table 1). On the other hand, the Cu concentration decreased in almost all treatments with other metals. It appears that numerous synergistic interactions and a few antagonistic interactions exist among different metals. Unfortunately, increasing the concentration of one metal often leads to an increase in the concentration of another, exacerbating the environmental pollution hazardous effects associated with heavy metals. Therefore, this is another additional and confirming argument for our hypothesis that soil contamination with Ni in this area (soil from vicinity of Ferronikeli Smelter in Drenas, Kosovo) has a very negative effect, even increasing uptake of other metals by plants.

3.2. Changes of chlorophyll pathway synthesis

The results of the activity of δ-aminolevulinic acid dehydratase (ALA-D) in the leaves indicated a strong significantly higher activity in maize seedlings that were grown in the non-polluted soil sample (control group, 8.18 μM PGB mg $prot.^{-1}$ h^{-1}) compared to those in the contaminated soil sample (5.52 μM PGB mg $prot.^{-1}$ h^{-1}) near the Ferronikeli Smelter in Drenas, Kosovo (Table 2). Similar results were obtained also when maize seedlings were cultivated under increasing metal concentrations (Ni, Cr and Pb); there was a significant decrease ($P < 0.05$) in the activity of ALA-D (δ-aminolevulinic acid dehydratase)

Table 2
Activity of δ-aminolevulinic acid dehydratase (ALA-D), δ-aminolevulinic acid (ALA), total chlorophyll content (Chl), reduced glutathione (GSH) and malondialdehyde (MDA) in leaves of maize seedlings from non-polluted location (control group) and polluted location (untreated soil and soil treated by salts of heavy metals Ni, Cr and Pb in different concentrations).

		ALA-D (μM PGB mg prot.$^{-1}$ h^{-1})	ALA (μM g^{-1} FW)	Chl (mg g^{-1} DW)	GSH (μM g^{-1} FW)	MDA (μM g^{-1} FW)
Control group		8.18^{A} ±0.63	25.00^{E} ±3.40	2.12^{A} ±0.01	1.91^{F} ±0.05	2.02^{E} ±0.13
Polluted soil	Untreated soil	5.52^{C-G} ±0.25	29.22^{C-E} ±32.45	2.06^{A} ±0.04	2.26^{E} ±0.10	2.19^{DE} ±0.10
	Ni 50 ppm	5.55^{C-G} ±0.18	31.16^{C-E} ±3.65	1.74^{DE} ±0.04	2.40^{E} ±0.09	2.14^{DE} ±0.03
	Ni 100 ppm	5.24^{D-G} ±0.29	31.85^{C-E} ±2.67	1.75^{C-E} ±0.02	2.55^{DE} ±0.10	2.40^{D} ±0.07
	Ni 200 ppm	4.39^{E-G} ±0.60	39.35^{BC} ±3.47	1.73^{DE} ±0.01	2.65^{C-E} ±0.08	3.52^{AB} ±0.21
	Ni 400 ppm	3.91^{G} ±0.28	50.57^{A} ±0.91	1.61^{F} ±0.03	3.25^{A} ±0.10	3.69^{A} ±0.08
	Cr 50 ppm	4.96^{AB} ±0.40	27.81^{DE} ±2.21	1.95^{B} ±0.01	2.40^{E} ±0.15	2.97^{C} ±0.04
	Cr 100 ppm	6.33^{A-E} ±0.63	42.91^{AB} ±4.98	1.97^{B} ±0.02	2.73^{CD} ±0.10	3.43^{AB} ±0.05
	Cr 200 ppm	4.70^{E-G} ±0.27	33.03^{C-E} ±4.61	1.79^{CD} ±0.02	2.92^{BC} ±0.03	3.55^{AB} ±0.02
	Cr 400 ppm	4.12^{FG} ±0.55	31.46^{C-E} ±1.44	1.69^{EF} ±0.02	3.27^{A} ±0.04	3.81^{A} ±0.05
	Pb 20 ppm	7.40^{A-C} ±0.39	30.80^{C-E} ±0.65	1.94^{B} ±0.02	2.46^{DE} ±0.11	2.44^{D} ±0.06
	Pb 50 ppm	7.02^{A-D} ±0.06	40.28^{BC} ±2.58	1.91^{B} ±0.02	2.68^{C-E} ±0.15	2.53^{D} ±0.16
	Pb 100 ppm	4.67^{E-G} ±0.30	37.40^{B-D} ±1.67	1.83^{C} ±0.04	2.93^{BC} ±0.09	3.20^{BC} ±0.26
	Pb 200 ppm	4.72^{E-G} ±0.34	37.68^{B-D} ±3.27	1.72^{DE} ±0.03	3.13^{AB} ±0.05	3.50^{AB} ±0.15
	F	5.82	5.22	25.80	15.46	24.57
	p < 0.05	0.000	0.000	0.000	0.000	0.000

Note. Soil from non-polluted location (control group) in Budakovë village, Suharekë municipality, Kosovo and polluted soil from the vicinity of Ferronikeli Smelter in Drenas, Kosovo. Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

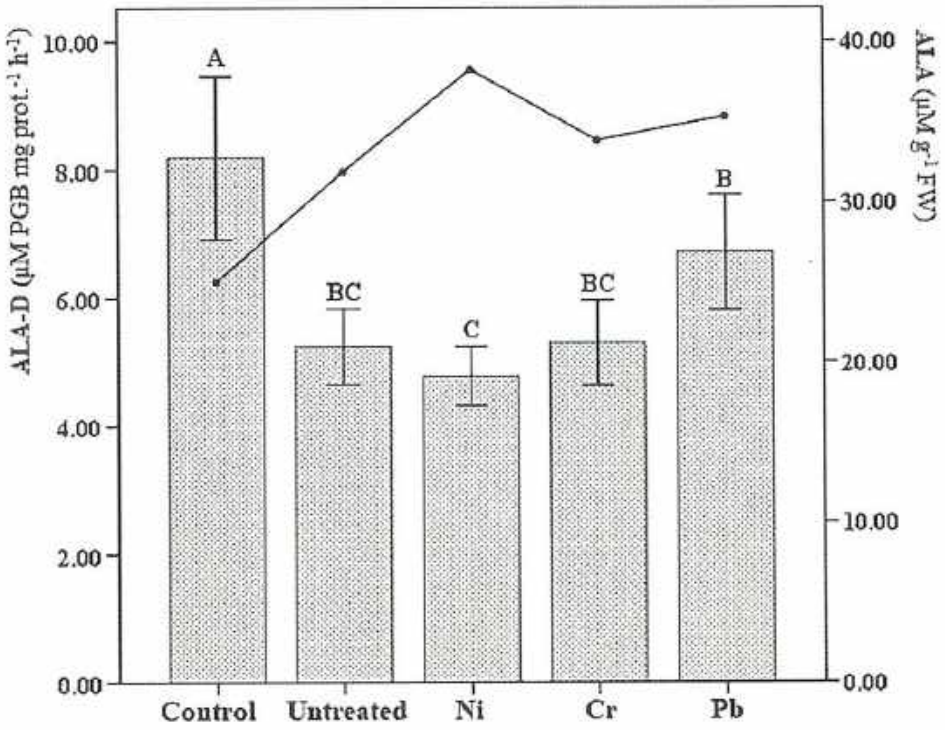

Fig. 1. Average of δ-aminolevulinic acid dehydratase (ALA-D) activity and δ-aminolevulinic acid (ALA) content were measured in maize seedlings from three groups: the control group, seedlings grown in untreated soil from a polluted location, and the mean of all concentrations for each metal treatment. Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

in the treatment leaves when compared to the control maize seedling leaves. Regarding the effect of metals on ALA-D activity, Ni and Cr exhibited stronger significant effects in inhibiting the activity of this enzyme, especially at high concentrations such as 400 ppm Ni and Cr, compared to maize seedlings from untreated soil samples. At these high concentrations of Ni and Cr, ALA-D activity was inhibited by approximately 50% compared to the control. Different effects of Pb were observed in maize seedlings. An increase in Pb concentration (100 and 200 ppm of Pb) in leaves had a negative impact on ALA-D activity. Conversely, maize seedlings treated with lower lead concentrations (20 and 50 ppm) exhibited higher ALA-D activity compared to all other metal treatments and the untreated soil. Similar results regarding the inhibitory effects of higher Pb concentrations were reported by Ahmed et al. (2020). They demonstrated that elevated Pb concentrations inhibited ALA-D activity and led to a significant decrease in total chlorophyll content in *Rhaphanus sativus* plants. Cenkci et al. (2010) observed a notable decrease in ALA-D activity in the leaves of fodder turnip when exposed to 5 mM Pb, and this decrease was statistically significant ($P \leq 0.05$) when compared to the control seedlings. They also found a negative correlation between the concentration of Pb and ALA-D activity in the leaves of fodder turnip.

In our results, there is a significant negative correlation between the concentration of Ni and the activity of ALA-D in maize seedlings, and a similar negative correlation trend is observed with Cr concentration (Table 4). In general, with results of the metal concentrations individually as averages alongside the average of ALA-D activity, it became evident that nickel had a significant negative impact on the activity of this enzyme compared to other metals, as well as untreated soil and control (Fig. 1). This association may also be linked to its higher concentration in leaves compared to other metals. Our results clearly demonstrate that ALA-D enzyme activity is highly sensitive to heavy metal pollution, even at lower concentrations. This sensitivity is attributed to its sulfhydryl nature, making ALA-D highly responsive to the presence of heavy metals. Furthermore, other studies on onion plants cultivated in industrial areas with higher lead concentrations in Mitrovica, Kosovo, have reported significant inhibition of ALA-D activity (Gashi et al., 2020). Moreover, according to other studies, ALA-D has been suggested as a highly suitable biomarker for biomonitoring soil pollution with heavy metals, particularly Ni (Osmani et al., 2018; Buqaj et al., 2023). Similar results for different heavy metal inhibitory effects of ALA-D activity, both *in vivo* and *in vitro* exposure to different metals, have been reported by other authors. Gupta et al. (2013) demonstrated that exposure of maize plants to Hg inhibited ALA-D activity, while Sarangthem et al. (2011) found that cadmium exposure led to enzyme inhibition and decreased total chlorophyll content in maize leaves. Exposure of *Cucumis sativus* plants to aluminum resulted in a significant inhibition of ALA-D activity (Pereira et al., 2006). In a study by Calgaroto et al. (2011) on *Pfaffia glomerata*, inhibition of ALA-D activity was observed in the presence of 50 μM Zn. Our results clearly demonstrate a concentration-dependent inhibition of ALA-D activity due to the accumulation of Ni and Cr in maize seedlings leaves. According to Osmani et al. (2018), tulip plants grown in soil with high concentrations of Ni exhibit varying effects on the activity of ALA-D, with enzyme activity decreasing as the concentration of Ni increases. It appears that the effect of these metals is directly related to the substitution of Mg with these metals, ultimately resulting in the inhibition of enzyme activity. The plant ALA-D enzyme contains Mg^{2+} ions in its octamer structure, and the binding of Mg^{2+} is relatively stable but can be removed from the enzyme by other metals with similar valence (Jaffe, 2000; Gashi et al., 2020). Furthermore, the enzyme ALA-D plays a crucial role in the synthesis of porphyrins, hemes, and chlorophylls, which are essential components for proper aerobic metabolism and photosynthesis (Pereira et al., 2006). The direct and rapid inhibition of ALAD activity by heavy metals binding to SH-groups on ALAD molecules results in a negative relationship between ALAD and PbB (Jaffe, 2000).

In contrast to ALA-D, the results of ALA content in leaves of maize seedlings (Table 2) showed significantly higher content in maize seedlings treated with various metal concentrations (Ni, Cr and Pb), compared to control group and untreated plants. Maize seedlings cultivated in treatments with escalating Ni concentrations, especially in 400 ppm, exhibited a significant increase ($P \leq 0.05$) in the level of ALA in leaves compared to the control treatment or other metal concentrations. Notably, there was a positive correlation between the concentration of Ni and the ALA level in maize seedlings (Table 3). Based on statistical analysis, the average concentrations of all metals and their effects on the ALA content revealed that Ni had the most significant impact, compared to Pb or Cr (Fig. 1). The increase in ALA content in treatments with metals corresponds to the low ALA-D enzyme activity. In cases where the enzyme activity was low, the ALA content increased, and vice versa (Fig. 1). The ALA-D enzyme catalyzes the asymmetric condensation of two molecules of ALA into porphobilinogen, and this enzymatic process results in a decrease in ALA content (Osmani et al., 2018; Siddiqui et al., 2020). We obtained a significant negative correlation between ALA and ALA-D activity in the leaves of maize seedlings and a positive correlation with total chlorophyll, as shown in Table 4. This is a clear indicator of the chlorophyll biosynthesis pathway and the significance of each step, as well as the stress-inducing effect of metals. Heavy metals disrupt the synthesis of ALA, which represents the initial

Table 3
The nuclear DNA content in picograms (pc) and cell cycle dynamics in the leaves of maize seedlings from non-polluted location (control group) and polluted location (untreated soil and soil treated by salts of heavy metals Ni, Cr and Pb in different concentrations).

		DNA content	Cell cycle		
		2C DNA pg	G1%	S%	G2/M%
Control group		5.16^{D} ±0.01	75.43^{A} ±0.42	4.00^{A} ±0.14	20.56^{B} ±0.28
Polluted soil	Untreated soil	5.23^{CD} ±0.05	66.31^{AB} ±6.05	3.15^{A} ±0.60	30.54^{AB} ±5.60
	Ni 50 ppm	5.64^{A} ±0.10	68.49^{AB} ±2.50	3.27^{A} ±0.91	28.23^{BC} ±3.07
	Ni 100 ppm	5.23^{CD} ±0.02	56.09^{A-C} ±5.93	4.44^{A} ±2.33	39.47^{A-C} ±3.61
	Ni 200 ppm	5.25^{CD} ±0.07	66.27^{AB} ±4.72	2.74^{A} ±0.79	30.98^{A-C} ±3.94
	Ni 400 ppm	5.41^{A-C} ±0.08	44.92^{C} ±7.26	4.85^{A} ±0.47	50.22^{A} ±6.93
	Cr 50 ppm	5.35^{B-D} ±0.08	59.24^{A-C} ±5.99	3.08^{A} ±0.74	37.67^{A-C} ±5.44
	Cr 100 ppm	5.39^{A-D} ±0.04	55.45^{BC} ±8.73	4.95^{A} ±1.00	39.59^{A-C} ±9.68
	Cr 200 ppm	5.20^{CD} ±0.09	61.12^{A-C} ±0.34	1.97^{A} ±0.52	36.91^{A-C} ±0.84
	Cr 400 ppm	5.53^{AB} ±0.05	53.93^{BC} ±1.77	2.77^{A} ±1.01	43.29^{AB} ±1.21
	Pb 20 ppm	5.47^{A-C} ±0.05	58.79^{A-C} ±5.76	2.86^{A} ±0.78	38.34^{A-C} ±4.99
	Pb 50 ppm	5.12^{D} ±0.03	54.56^{BC} ±1.13	3.05^{A} ±0.89	42.38^{AB} ±0.41
	Pb 100 ppm	5.22^{CD} ±0.07	49.86^{BC} ±12.72	3.44^{A} ±0.68	46.69^{AB} ±13.40
	Pb 200 ppm	5.27^{B-D} ±0.05	55.70^{BC} ±4.11	3.01^{A} ±0.48	41.28^{AB} ±4.06
	F	3.44	1.87	0.86	1.84
	$p < 0.05$	0.004	0.087	0.591	0.093

Note. Soil from non-polluted location (control group) in Budakovë village, Suharekë municipality, Kosovo and polluted soil from the vicinity of Ferronikeli Smelter in Drenas, Kosovo. Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

Table 4
Correlation between heavy metals in leaves of maize seedlings, biochemical and molecular parameters.

	ALA-D	ALA	GSH	MDA	Chl	cDNA	G1	S	G2/M
Fe	-0.425**	0.032	0.392**	0.445**	-0.388**	0.322**	-0.136	-0.113	0.155
Cu	0.340**	-0.159	-0.312*	-0.419**	0.570**	-0.277*	0.265*	-0.207	-0.242
Mn	-0.109	-0.206	0.223	0.287*	-0.062	0.121	0.089	-0.167	-0.068
Cr	-0.227	-0.152	0.351**	0.373**	-0.205	0.278*	-0.074	-0.172	0.099
Ni	-0.392**	0.526**	0.356**	0.362**	-0.502**	0.099	-0.313*	0.252*	0.285*
Pb	0.161	-0.035	0.321**	0.249*	-0.172	-0.200	-0.197	-0.081	0.212
ALA	-0.327**								
GSH	-0.432**	0.342**							
MDA	-0.359**	0.334**	0.684**						
Chl	0.530**	-0.292*	-0.620**	-0.479**					
cDNA	-0.147	0.035	0.074	-0.014	-0.182				
G1	0.179	-0.319**	-0.491**	-0.421**	0.367**	-0.032			
S	0.082	0.150	-0.038	-0.066	0.052	0.010	-0.209		
G2/M	-0.194	0.305*	0.506**	0.438**	-0.381*	0.032	-0.991**	0.076	

** Correlation is significant at the 0.01 level
* Correlation is significant at the 0.05 level.

step in the biosynthesis of tetrapyrrole, ultimately leading to heme production (Vajpayee et al., 2000). However, it is worth noting that the biosynthesis of chlorophyll may not solely depend on ALA synthesis but could also be influenced by the activity of ALA-D. Changes in ALA-D activity have been linked to a reduction in chlorophyll content in many terrestrial plants exposed to various concentrations of Pb, Cd, and Hg (Panda et al., 2020). Our results are consistent with the results of other researchers for ALA content. Vajpayee et al. (2000) reported an increase in ALA content in *Nymphaea alba* plants under the influence of chromium, along with a decrease in protein and total chlorophyll content. Similarly, Cenkci et al. (2010) observed an increase in ALA content in *Brassica rapa* L. plants after exposure to lead (Pb). Accumulation of ALA due to heavy metal effects on ALA-D activity were also reported for maize plants (Buqaj et al., 2023; Gupta et al., 2013), onion plants (Gashi et al., 2020), ramonda plants (Gashi et al., 2019), and tulipa plants (Osmani et al., 2018).

Chlorophyll, as a vital biological molecule, plays a fundamental role in photosynthesis by capturing energy-rich photons to synthesize carbohydrates. Due to its delicate nature, chlorophyll is highly sensitive to environmental stresses. In this study, we discovered that chlorophyll content in leaves of maize seedlings had a significant reduction in all metal treatments groups compared to the control (Table 2). In all cases, as the metal concentration dose increased, the chlorophyll content continuously decreased, particularly in the treatments with 400 ppm Ni and Cr. Furthermore, we observed a strong significant positive correlation between total chlorophyll and ALA-D and a significant negative correlation between Ni and ALA (Table 4). These results of correlation may be a consequence of the direct impact of heavy metals on their synthesis; on the other hand, it was also the result of inhibition of the initial pathway of porphobilinogen biosynthesis where the activity of the ALA-D enzyme was inhibited by these metals. It appears that ALA-D activity was inhibited by metal toxicity, resulting in the buildup of ALA and reduced chlorophyll content. This assumption holds even when comparing total chlorophyll content in the control group of maize seedlings with untreated soil from the polluted area, where no significant difference was observed (2.06 and 2.12 mg g^{-1} DW, respectively). The low concentration of metals in the leaves did not affect the chlorophyll content, but only that of ALA-D, which did not result in a significant effect. Similar results have been reported from other authors. Cenkci et al. (2010) reported that content of photosynthetic pigments in fodder turnip leaves decreased substantially ($P \leq 0.05$) with the increase of Pb concentration. Reduction in chlorophyll content by excess of heavy metals has been reported in maize plants after exposure to agricultural soil contaminated with Ni (Buqaj et al., 2023). The decreases in chlorophyll could be due to suppression of chlorophyll biosynthesis and/or accelerated chlorophyll degradation. ALA is an essential precursor of chlorophyll, and overproduction of ALA in transgenic canola (*Brassica napus*) promoted leaf chlorophyll accumulation (Sun et al., 2015). Positive significant correlation between total chlorophylls with MDA and GSH was recorded in leaves of maize seedlings (Table 4). Furthermore, the decline in chlorophyll content and its degradation on the maize seedlings may be attributed also to the excessive production of reactive oxygen species (ROS) and lipid peroxidation triggered by the toxicity of metals in higher doses (Tables 2 and 4). Moreover, ROS are generated as part of the photosynthetic process and can lead to the destruction of chlorophyll (Triantaphylides and Havaux, 2009).

3.3. Oxidative stress level

Based on our results, when maize seedlings were grown in the presence of different concentrations of Ni, Cr and Pb, the levels of oxidative stress increased, leading to significantly higher production of GSH and elevated levels of lipid peroxidation (MDA) (Table 2). Under non-stress conditions (control group), maize seedlings produced lower levels of GSH (1.91 μM g^{-1} FW) and MDA (2.02 μM g^{-1} FW) compared to slightly stressed conditions in untreated soil from polluted regions (2.26 and 2.19 μM g^{-1} FW, respectively) and treatments with heavy metals. In all treatments of maize seedlings with high concentrations of metals (Ni and Cr 400 ppm; Pb 400 ppm, respectively), the amount of GSH and MDA was significantly higher than in the lower concentrations. Especially at concentrations of 400 ppm for Ni and Cr, the levels of GSH and MDA in the leaves of maize seedlings nearly doubled compared to the control group. This is further supported by correlations that indicate a strong positive relationship between the levels of GSH and MDA with Ni and Cr concentration in the leaves of maize seedlings (Table 4). On the other hand, when considering the average amount of GSH in all treatments with individual metals, no significant differences were found among the metals. However, there was a higher tendency for chromium to influence the increase in GSH levels (Fig. 2). Glutathione plays a crucial role in the Ascorbate-Glutathione (AsA-GSH) cycle, where it serves as a vital component responsible for scavenging various free radicals and helping to maintain the cellular redox balance (Hasanuzzaman et al., 2020). Moreover, GSH is crucial for heavy metal detoxification, including metals like cadmium and nickel. Additionally, it serves as a precursor for the synthesis of phytochelatins, which are important molecules involved in the sequestration and detoxification of heavy metals in plants (Mansoor et al., 2023). Khan et al. (2016) reported an increase in GSH levels as the concentration of Ni increased in Brassica juncea, and this observation aligns with the idea that GSH plays a vital role in heavy metal detoxification in plants. In terms of MDA in our experiment with maize seedlings, it is evident that, overall, Cr had a significantly more toxic effect on lipid peroxidation compared to other metals (Fig. 2). In general, it is evident that maize seedlings exhibit a higher level of cellular oxidative stress, primarily induced by chromium.

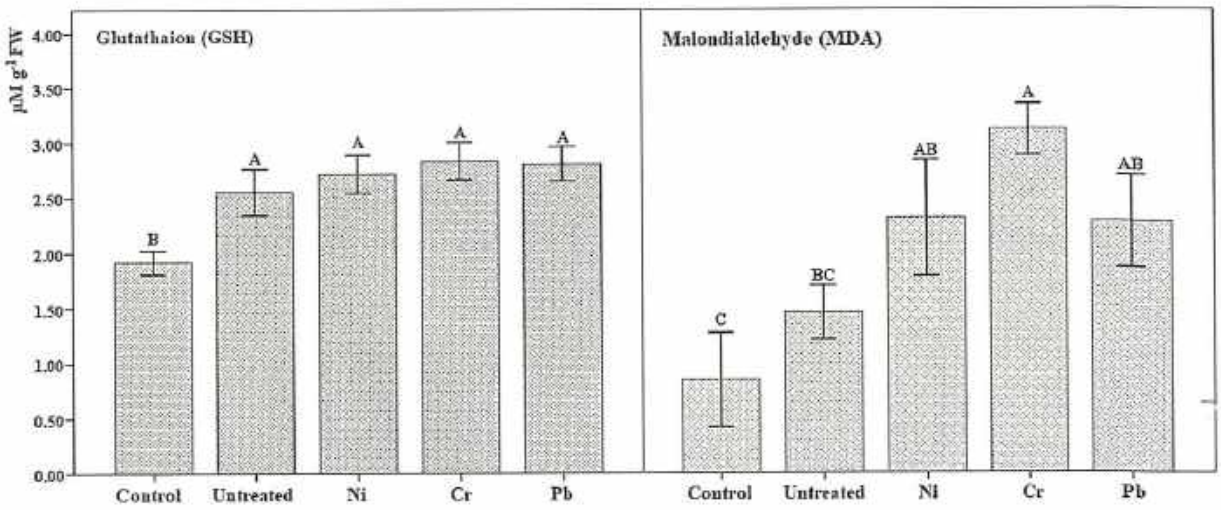

Fig. 2. Average of glutathione (GSH) and malondialdehyde (MDA) content were measured in maize seedlings from three groups: the control group, seedlings grown in untreated soil from a polluted location, and the mean of all concentrations for each metal treatment. Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

Therefore, an elevation in the levels of specific parameters, including ROS (such as H_2O_2, O_2^-), electrolyte leakage and MDA, arising from the peroxidation of polyunsaturated fatty acids, can serve as valuable indicators of oxidative stress. This, in turn, can be indicative of cellular damage and the potential for cell death (Siddiqui et al., 2020). In our maize seedling experiment, we observed an increase of MDA, ALA and GSH in all heavy metal treatments; this increase is indicative of oxidative stress, which can lead to cellular dysfunction. This association between heavy metals and oxidative stress has already been described in maize (Buqaj et al., 2023; Requejo and Tena 2005) and other plant species, such as Arabidopsis (Piacentini et al., 2020), tulipa (Osmani et al., 2018), onion (Gashi et al., 2020), and garlic (Ruiz-Torres et al., 2017). Our results for higher MDA content in leaves of maize seedlings treated with Cr and Ni are also in accordance with other authors. Georgiadou et al. (2018) reported that Ni in higher concentration (500 ppm) significantly increased MDA content in leaves of basil. Juknys et al. (2012) have reported similar findings for effects of Ni at 1000 ppm concentration on MDA in spring barley.

3.4. DNA content and cell cycle dynamics

The 2C DNA content in the leaf cells of maize seedlings exhibited significant differences in some cases between the control group and the maize seedlings treated with heavy metals (Ni, Cr, and Pb), as shown in Table 3. Specifically, the DNA content varied significantly, with values ranging from 5.20 pg in treatments with 200 ppm of Cr to 5.64 pg in treatments with 50 ppm of Ni, compared to the maize seedlings from the control group, which had a DNA content of 5.16 pg (Table 3). Among the metal treatments, DNA content was higher in maize seedlings treated with different concentrations of Ni and Cr. In addition, there was significant correlation between Cr concentration in leaves and DNA content (Table 4). In general, based on these DNA content results, there is a trend of increased DNA content in the leaf cells of maize seedlings treated with metals compared to untreated plants. Furthermore, given the advantages of directly measuring the effects of genotoxic chemicals on DNA, particularly their sensitivity and short response time, numerous studies have demonstrated a relationship between heavy metals and genome size. Similar interesting results for increasing DNA content in plants exposed to heavy metals was reported from Citterio et al. (2003). Their hypothesis suggests that the observed increase in DNA content may be a part of the plant's strategy to combat and adapt to heavy metal toxicity. Another argument for increased DNA content was reported at found significant changes in DNA content in *Viola tricolor* grown under natural conditions in soil polluted with metals (Zn, Pb, Cu, Cd) compared to non-polluted soil (Slomka et al., 2011). The DNA content increased with increasing of Cd concentration at *Thlaspi caurlescens* (Monteiro et al., 2010). These researches indicate an increase in the amount of DNA as a strategy and protective mechanism against the toxicity of heavy metals by activating specific genes for this purpose.

To analyze cell cycle progression in the leaves of maize seedlings, we examined cell cycle arrest from plants grown in the soil treated with various concentrations of Ni, Cr, and Pb under stress using flow cytometry. The cell cycle dynamics results for control maize seedlings for G1 and S phase were significantly higher (75.43% of cells were in G1 stage and 4.00% in S stage), compared to maize seedlings from polluted soil and all heavy metal treatments (Table 3). In contrast, the G2/M phase in control seedlings was significantly lower (20.56%), compared to maize seedlings from polluted soil and all heavy metal treatments. In general, all metals had a negative impact on the G1 and G2/M phases of the cell cycle, while the S phase remained unaffected (Fig. 3). Specifically, the G1 phase exhibited a significant decrease relative to the control, while the G2/M phase showed a significant increase. As shown in Table 3, the proportion of cells in the G1 phase was higher in the control seedlings. However, heavy metal stress significantly altered this proportion, especially with Ni at 400 ppm, where only 44.92% of nuclei were in this phase. These pronounced effects on cell cycle arrest were also observed in the same treatment for the G2/M phase, where 50.22% of nuclei remained in this phase. Furthermore, there was a negative correlation between Ni concentration in leaves and cells in G1 phase, whereas there was a positive correlation with S and G2/M phases (Table 4). Although there were no significant changes in the S phase between all treatments, an increasing trend was observed in the difference between Ni and Cr treatments. In the high-concentration Ni treatments, the S phase reached up to 4.85%, indicating more delays in the G1/S phase of the cell cycle, whereas, in the high-concentration Cr treatments, the S phase was lower (2.77%), indicating more delays in the G2/M phase of the cell cycle. These findings provide support for the hypothesis that exposure to heavy metal stress can lead to alterations in the expression of cell cycle regulatory genes that play roles in the transitions between G1/S and G2/M phases in the leaves of maize seedlings. This suggests that heavy metal stress can influence plant cell cycle regulation at the molecular level. Similar results for negative effects of heavy metals in cell cycle progression have been reported from other authors. Cui et al. (2017) investigated the effect of different Cd concentrations on *Arabidopsis thaliana* and observed a decrease in the proportion of cells in the G1 phase and an increase in the G2/M phase as

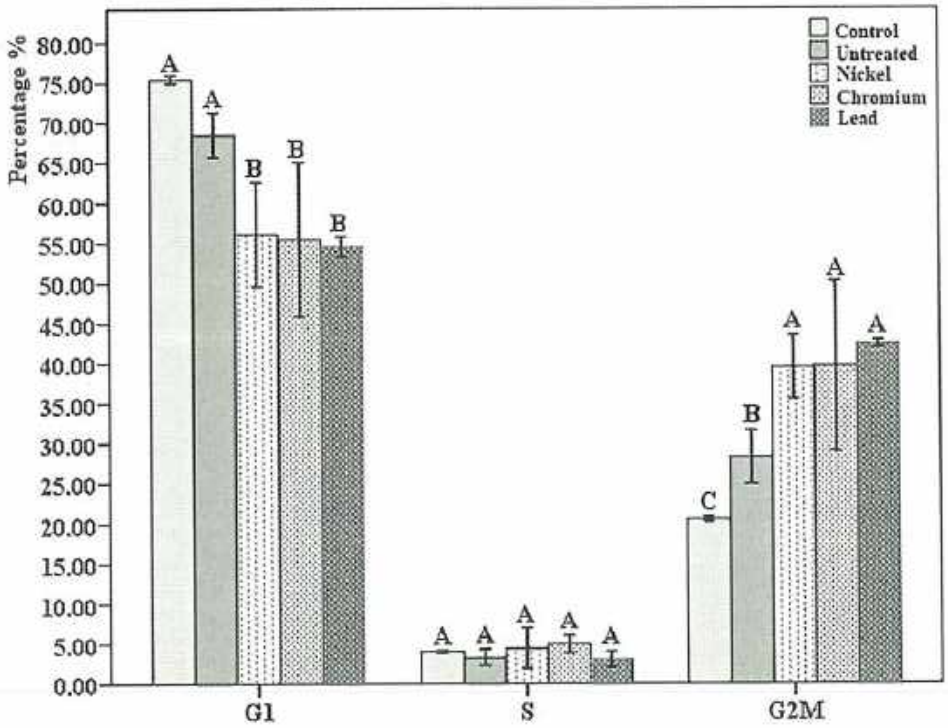

Fig. 3. Average of G1, S and G2M cell cycle dynamics were measured in maize seedlings from three groups: the control group, seedlings grown in untreated soil from a polluted location, and the mean of all concentrations for each metal treatment. Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

the Cd concentration increased. Rodriguez et al. (2019) conducted a study on pea under abiotic stress induced by different Pb doses and observed similar shifts in cell cycle distribution, with decreasing G1 phase and increasing G2/M phase proportions. Youssef et al. (2021) discovered an increased distribution of cells in the G2 phase in maize after exposure to Cd. Furthermore, cell cycle checkpoints and DNA repair control mechanisms are crucial factors that play a significant role in regulating cell cycle progression in plants when they are exposed to DNA-damaging agents, as highlighted by Cao et al. (2018). These mechanisms are essential for maintaining genomic integrity and ensuring that cells respond appropriately to DNA damage, which is critical for the overall health and survival of plants under such stress conditions. This observation suggests that cells may have been arrested at the G1/S checkpoint, which allows them to undergo DNA damage repair before progressing into the S-phase. This regulatory mechanism aligns with the findings reported by Deckbar et al. (2011).

4. Conclusions

This research presents novel findings into oxidative stress levels, chlorophyll biosynthesis pathway suppression and cell cycle arrest, resulting from the exposure of maize seedlings to three heavy metals (Ni, Cr and Pb) in four different concentrations in maize seedlings, when applied as additional doses to contaminated soil from the vicinity of Ferronikeli in Drenas, Kosovo. Our findings indicate that maize seedlings have accumulated significantly higher concentrations of Ni and Cr in their leaves when the metals were applied in soil compared to control, and their concentrations in leaves corresponded to applied concentrations in the soil. Furthermore, there are some synergistic relationships between these metals and Fe and Mn concentrations in leaves. The accumulation of heavy metals, especially Ni and Cr at 400 ppm, had significant negative effects on ALA-D activity, with inhibition up to 50%, and a significant decrease in total chlorophyll content, compared to control plants. Cell cycle dynamics are significantly altered, with G1 and G2/M phases almost equal at higher Ni and Cr concentrations, indicating cell cycle arrest. Moreover, as heavy metal concentrations increase, there is a corresponding escalation in cellular damage, emphasizing the concentration-dependent relationship between metal exposure and harm to plant cells. Ni is identified as the primary cause of cellular damage, with a concentration-dependent relationship between metal exposure and harm to plant cells.

The study concludes that biomarkers such as ALA-D activity, ALA, GSH, MDA levels, and cell cycle dynamics are highly sensitive indicators for monitoring pollution levels, especially heavy metals, and their effects on ecosystem components. Further research may be necessary to explore the molecular mechanisms underlying these responses and to devise strategies for mitigating the adverse effects of heavy metal contamination in this agricultural area.

CRediT authorship contribution statement

Bekim Gashi: Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft. **Liridon Buqaj:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Ramë Vataj:** Formal analysis, Validation, Writing – review & editing. **Metin Tuna:** Formal analysis, Investigation, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100379.

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WE HEREBY CERTIFY THAT THE FOLLOWING NAMELY ONE

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have participated and presented a paper titled

ANTIOXIDANT RESPONSE OF MAIZE (ZEA MAYS L.) DUE TO SOIL CONTAMINATION BY HEAVY METALS IN THE VICINITY OF THE FERRONIKEL SMELTER IN DRENAS, KOSOVO

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ANTIOXIDANT RESPONSE OF MAIZE (ZEA MAYS L.) DUE TO SOIL CONTAMINATION BY HEAVY METALS IN THE VICINITY OF THE FERRONIKEL SMELTER IN DRENAS, KOSOVO.

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The contamination of agricultural soils with heavy metals as a result of industrial activity is worrying for biota in general. Chemical elements or more specifically heavy metals have the ability to be accumulated in plants and are associated with various effects of a toxic nature. The same chemical elements are transferred to humans and animals through the food chain. The purpose of this study was to analyze the antioxidant response in the maize plant by measuring total antioxidant capacity (TAC), reduced glutathione (GSH), and the level of lipid peroxidation through the product known as malondialdehyde (MDA). The total antioxidant capacity, MDA and GSH were determined and analyzed by applying the standard protocols in the leaves of the maize plant (Zea mays L.). Firstly, the concentration of some heavy metals (Pb, Cr, Ni, Fe, Cd, Cu, Mn) was determined from the soil samples and also the effect these elements have in relation to oxidative stress in the maize plant which is cultivated in the soil taken from the agrocultural surfaces near the Ferronikel smelter in Drenas, compared to an area which is clean from the environmental aspect. Relatively high values were found in some locations around the smelter, especially for nickel (809.46 mg/kg) and chromium (801.10 mg/kg). In general, the values of MDA and GSH marked a significant increase in relation to the concentration of heavy metals, as well as the values of the total antioxidant capacity marked an increase. Our study clearly showed that exposure to heavy metals generates oxidative stress. The maize plant represents a suitable model for the research of biochemical parameters that are related to oxidative stress, respectively the response at the molecular level that is generated in the plant as a result of its development in substrates that have different concentrations of heavy metals.

Keywords: Agricultural Soil, Heavy Metals, Maize, Total Antioxidant Capacity, MDA,GSH.

PBE PLANT BIOLOGY EUROPE 2023 July 3 – 6, 2023 MARSEILLE

CERTIFICATE OF ATTENDANCE

This document is to certify that

Liridon Buqaj

has participated to the Plant Biology Europe meeting which took place at the Palais du Pharo in Marseille (France) **from July 3 to 6, 2023.**

Marseille, 6 July 2023

On behalf of the PBE Meeting 2023
Organization Committee

FESPB Federation of European Societies of Plant Biology

Christophe Robaglia, Professor Aix Marseille University, President FESPB
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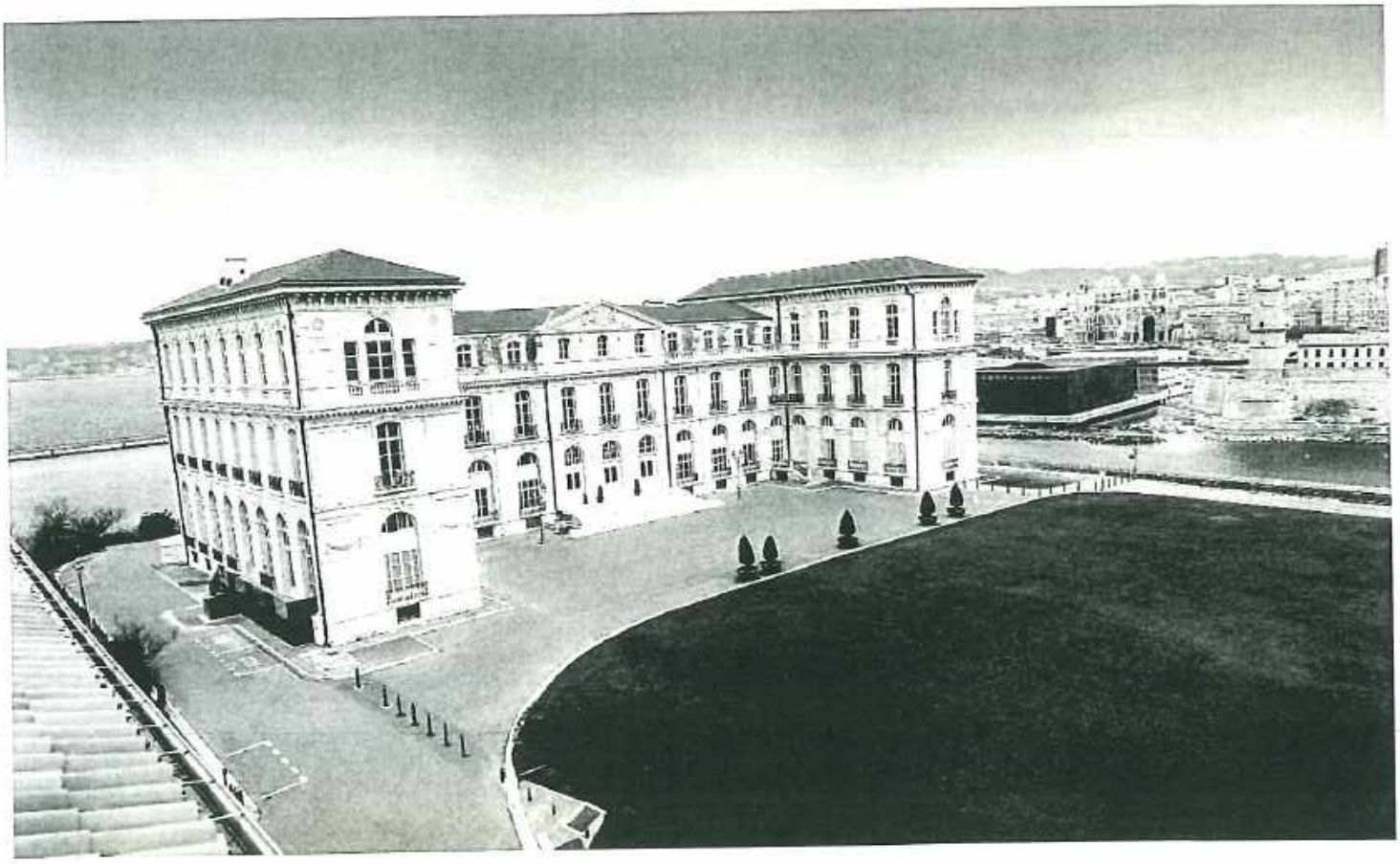

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INDEX

INTRODUCTION

The Organizing Committee, the Scientific Committee, the Federation of the European Societies of Plant Biology, the French Society of Plant Biology and the Biosciences and Biotechnology Institute of Aix-Marseille welcome you to Plant Biology Europe.

This international meeting covers a wide range of Plant Science topics across multiple disciplines and at different scales.

Among the many different themes that are being addressed during the meeting, a particular emphasis is placed on plants and climate changes, algal biology and bioenergy.

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the plant, development using a combination of biochemical, molecular-biological, genomic, and bioinformatic approaches, we also investigate whether there are any additional biochemical or functional similarities between these putative Arabidopsis and human homologs.
This work was supported by Czech Science Foundation (21–28265S).

0143-C
GUN1 INVOLVEMENT IN THE REDOX CHANGES OCCURRING DURING CHLOROPLAST DEVELOPMENT AND HEAT STRESS RESPONSE

Maria Concetta DE PINTO[1]*; Stefania FORTUNATO [1]; Cecilia LASORELLA [1]; Nunzio DIPIERRO [1]; Nicolaj JERAN [1]; Federico VITA [1]; Paolo PESARESI [1]

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Plant development and response to environmental changes require a mutual communication between plastids and the nucleus. By retrograde signals, plastids transmit information about their functional and developmental state to adjust nuclear gene expression. GENOMES UNCOUPLED 1 (GUN1), a chloroplast-localized protein, acts as one of the main players of retrograde signaling. Redox changes greatly influence plant response to endogenous and environmental stimuli. We focused on the interplay between GUN1 and redox regulation during plastid biogenesis and heat stress response (HSR). Arabidopsis wild type (wt) and gun1 seedlings, were grown for six days in presence or absence of lincomycin, which perturbs chloroplast development. To study HSR, 15-day-old wt and gun1 plantlets grown at 22°C were exposed to 3 hours of heat stress (HS) at 45°C. Results indicate that in response to both lincomycin and HS, GUN1 is required for the redox-dependent plastid-to nucleus communication.

0144-A
DNA CONTENT AND CELL CYCLE ON MAIZE PLANT (ZEA MAYS L.) UNDER HEAVY METALS STRESS

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Heavy metals affect organisms at the molecular level, showing various abnormalities. The purpose of this study is to determine the effects of heavy metals on DNA content and cell cycle phases (G1, S, G2M), in the leaves of the maize plant. Maize plants were treated with a certain concentration of metals: Ni and Cr (50ppm, 100ppm, 200ppm, 400ppm), and Pb (20ppm, 50ppm, 100ppm, 200 ppm), separately. The analysis of the samples was carried out by flow cytometry method. Our results indicate small changes of the DNA content caused by

the increasing concentration of the metals compared to the control group, as well as in the cell cycle phases, depending on the variations of the metals and concentration levels with a decrease in the distribution of the cells in the G1 phase and an increase in G2M. Our study shows that the maize plant can be used as a model to evaluate the effect of Ni, Cr and Pb, depending on their concentration in relation to the DNA content and cell cycle phases.

0145-B
ALTERATIONS IN SPECIALIZED METABOLITES' PROFILE OF DAUCUS CAROTA L. CALLI INDUCED BY LOW-TEMPERATURE PLASMA TREATMENT

Suzana ZIVKOVIC[1]*; Milutinovic MILICA [1]; Danijela MISIC [1]; Nakarada DURA [2]; Mojovic MILOS [2]; Olivera JAVANOVIC [3]; Nikola SKORO [3]; Nevena PUAC [3]

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Non-thermal plasma (NTP) technology offers a promising future in plant metabolic engineering, being energy efficient and Eco-friendly alternative to the conventional treatments [1]. Plasma environment is enriched with reactive oxygen and nitrogen species (RONS) that participate in various signaling pathways in plants by regulating their metabolic and developmental processes. In the present study calli of different carrot (Daucus carota L.) varieties was treated by using plasma needle device designed for biomedical applications [2]. Metabolite profiling revealed that plasma treatment could induce severe qualitative and quantitative changes of the major phenolic compounds detected in carrot calli. Current metabolic alteration was followed by the significant shift in the antioxidant capacity of the treated calli. Obtained results outline the potential application of plasma treatment as a novel elicitor for the production of bio-active compounds in plant in vitro culture systems.

0146-C
COLD STRESS TOLERANCE IN FLAX (LINUM USITATISSIMUM L.): CHARACTERIZATION AT THE PHYSIOLOGICAL, METABOLIC, TRANSCRIPTOMIC AND GENETIC LEVELS.

Henri DESAINT[1]*; Adèle DE GIULI [1]; Hanine IDELBI [2]; Klara CIK [1]; Jean-Xavier FONTAINE [1]; Roland MOLINE [1]; Emmanuel PETIT [1]; Solène BASSARD [1]; Romain ROULARD [1]; Damien HERFURTH [1]; Hervé DEMAILY [3]; Stéphanie GUENIN [3]; Laurent GUTIERREZ [3]; David MATHIRON [4]; Nicolas MONTRELAY [5]; David GAGNEUL [1]; Christophe PINEAU [2]; Gaëlle MONGELARD [3]; Anthony QUERO [1]

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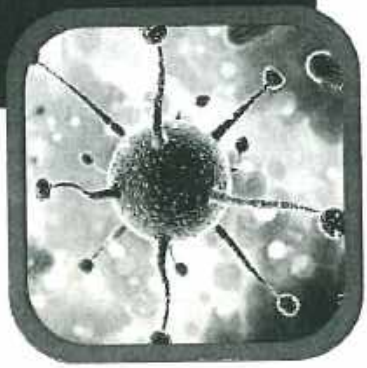

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Welcome

Dear colleagues and friends,

We are delighted to invite you to the 8th International Congress on Applied Biological Sciences 2023 (ICABS-2023) that will take place in Pristina, Republic of Kosovo, from September 13th to 16th. This congress was organized by researchers from Eskisehir Osmangazi University and the University of Pristina "Hasan Pristina".

Our field is developing extremely fast with important implications for basic science, biology, and medicine. ICABS-2023 aims to provide an interdisciplinary platform for significant scientific and professional activity in research findings, developments, and applications.

The scientific program includes invited international experts, selected presentations for young investigators, educational sessions, and poster presentations. Thus, the congress is a unique opportunity to get inspired for scientific work, share your results, and shape the future of the field.

ICABS-2023 will be a compact meeting and will be including some novel approaches to help make it extra rewarding and enjoyable, such as a 'stand-up' social evening for those interested in applied biological sciences.

This congress will serve as an excellent platform for participants to broaden their knowledge, enhance their professional networks, and gain new insights into the latest trends and developments in the basic sciences. For the ICABS-2023, we welcome researchers at all career stages, from a range of research fields to gather together in the Applied Biological Sciences for a better future.

We are looking forward to meeting you in Kosovo!

Prof. Dr. Didem TURGUT COŞAN
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POSTER PRESENTATION

8th INTERNATIONAL CONGRESS on APPLIED BIOLOGICAL SCIENCES
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EFFECT OF DIFFERENT CONCENTRATIONS OF Ni, Cr AND Pb ON SOME BIOCHEMICAL PARAMETERS AND ANTIOXIDANT RESPONSE OF MAIZE (*Zea mays* L.)

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Abstract

Heavy metals are normal components of soil and some of them are essential, but an increase in their concentration in soil directly attacks plants and causes phytotoxicity. The objective of this study is to evaluate the toxic effects of different concentrations of Ni, Cr and Pb by measuring some biochemical biomarkers and some indicators of the degree of oxidative stress in maize plants. Maize plants were grown in vegetation rooms and the substrate in which they were planted was separately poisoned with different concentrations of the metals Ni and Cr (50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg) and Pb (20 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg). All the parameters listed below were evaluated in the leaves of maize plants using appropriate methods. In general, with the increase in the concentrations of Ni, Cr and Pb, the decrease in the activity of δ-aminolevulinic acid dehydratase and total chlorophyll content, the increase in aminolevulinic acid content, the increase in glutathione and malondyaldehyde level were studied. Changes were also observed in total antioxidant capacity depending on the type of metal and exposure dose. Nickel, chromium, and lead have a toxic effect on the maize plant proportional to the increase in their concentration, decreasing the activity of the enzyme δ-aminolevulinic acid dehydratase and, consequently, the content of total chlorophylls. Under the stress of these metals, free radicals are formed in the plants, causing lipid peroxidation (formation of malondyaldehyde), and the enzymatic and non-enzymatic antioxidant protection mechanisms are activated.

Keywords: Ni, Cr, Pb, maize, biochemical parameters, antioxidant response