"AUREL VLAICU" UNIVERSITY OF ARAD
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DOMAIN: ENVIRONMENTAL ENGINEERING

ABSTRACT

Ph.D. Thesis

Studies on the influence of medicinal drugs present in the soil on plants

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CONTENTS

INTRODUCTION

Due to conventional processes used in wastewater treatment plants are not designed to remove micropollutants, they can persist in treated wastewater effluent.

Many of these micropollutants may therefore be present in aquatic environments, including surface waters, and their presence in surface waters poses a threat to ecosystems and thus to human health.

The presence of micropollutants in the aquatic environment is associated with various adverse effects, including short- and long-term toxicity and antibiotic resistance in microorganisms.

The physical properties of the micropollutant can affect their movement from one phase to another (e.g., soil-water transfer). The mobility of compounds is determined by transport/retention factors, which depend on chemical properties such as acidity constant (pKa) and octanol-water partition coefficient (Kow).

These micropollutants include nonylphenol and nonylphenol ethoxylates, bisphenol A, pharmaceuticals, personal care products, and steroid hormones.

In an age of high standards of human health care, many pharmaceuticals are routinely used to cure or prevent other diseases such as headaches, muscle aches, or inflammatory conditions. Over-the-counter medicines are common, especially monocyclic and polycyclic non-steroidal anti-inflammatory drugs (NSAIDs) without a prescription.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used pharmaceuticals internationally and are among the top 10 persistent pollutants.

Among NSAIDs, diclofenac, indomethacin, and naproxen are the most commonly detected in the environment and are recognized as potential contaminants with a high water-octanol partition coefficient and low pKa values.

In the same direction, these pharmaceuticals show a high capacity for passive diffusion through biological membranes, with high persistence in aquatic environments.

Because of the relatively low presence of NSAIDs in groundwater, there are limited studies and practices concerning the occurrence of these compounds in groundwater. However, there are significant sources of contamination, such as leachate from landfills and interaction with water bodies and sewage system drainage, which can affect groundwater.

NSAIDs are mobile pharmaceuticals that leach from solid waste, sewage sludge and fertilizers and eventually end up in groundwater.

From these waters, these contaminants can reach the root system of plants and thus be a stress factor for them.

Thus, plants are subject to stressors that can lead to changes in their primary and/or secondary metabolism.

Bean, pea, chickpea, lentil, and cowpea plants belong to the *Fabaceae*, the third largest flowering plant family.

In addition, no other family has a wider geographical distribution in the broader range of habitats distributed on all continents except Antarctica.

OBJECTIVES

This Ph.D. thesis aims to investigate how non-steroidal anti-inflammatory drugs (NSAIDs) in soil Influence the development of plants of the *Fabaceae* family. The research is based on the hypothesis that in the absence of advanced methods to break down these drugs, significant concentrations of NSAIDs exist in groundwater and/or soil. These compounds can reach the reticular system of plants and are assimilated by the plants and involved in their metabolic cycles through this pathway.

The paper aims to:

- Determining the impact of non-steroidal anti-inflammatory drugs on plant development.
- Determination of concentrations that can influence the values of photosynthesis parameters and plant metabolism compounds.
- Evaluation of photosynthesis parameters, chlorophyll and carotene concentrations, emission of volatile organic compounds for *Fabaceae* plants under NSAID-induced stress.
- Establishment of species less sensitive to micropollutants in soil.
- Assessing the environmental implications of volatile organic compound emissions from abiotically stressed plants.

CHAPTER 1

1. Non-steroidal anti-inflammatory drugs (NSAIDs)

1.1. Generalities

Pharmaceuticals are a group of emergent organic compounds that have enhanced our quality of life. The pharmaceutical industry is responsible for developing, producing, and marketing branded and generic pharmaceuticals. In 2014, total pharmaceutical revenues exceeded 1 trillion United States dollars (USD) for the first time.

The market has been growing at an annual rate of 5.8% since 2017. In 2017, worldwide pharmaceutical market revenue was USD 1143 billion and will reach 1462 billion USD in 2021 [1]. The largest fraction of these revenues corresponds to North America due to the leading role of the US pharmaceutical industry. However, the Chinese pharmaceutical sector has shown the highest growth rates worldwide [1].

Several factors such as reduced taxes and lowered drug prices in the US, a gross domestic product growth greater than 6% in China and India, widespread population aging and sedentary lifestyles leading to increase chronic disease, and industrialized data services in research and development (R&D) enabling the use of clinical trial data in trial simulations, lowered regulatory barriers for new drugs in the US, and high urban pollution levels increasing the incidence of conditions such as asthma are driving healthcare market growth [1].

Analgesic drugs include non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol (acetaminophen). NSAIDs are used to relieve pain and also to suppress inflammation in a similar way to steroids but without their side effects. Paracetamol, however, has no anti-inflammatory properties.

Anti-inflammatories, analgesics, and antipyretics are not chemically related but share specific therapeutic actions. The primary mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase (COX). Cyclooxygenase is required to convert arachidonic acid into thromboxanes, prostaglandins, and prostacyclins.

The therapeutic effects of NSAIDs are attributed to the lack of these eicosanoids.

Specifically, thromboxanes play a role in platelet adhesion. Prostaglandins cause vasodilation and increase the temperature set point in the hypothalamus.

There are two isoenzymes of cyclooxygenase, COX-1, and COX-2. COX-1 is constitutively expressed in the body and plays a role in maintaining gastrointestinal mucosa, renal function, and platelet aggregation. COX-2 is not constitutively expressed in the body. Instead, it is induced during an inflammatory response.

Most NSAIDs are non-selective and inhibit both COX-1 and COX-2. However, COX-2 selective NSAIDs (e.g., celecoxib) only target COX-2 and therefore have a different side effect profile. It is important to note that COX-1 is the primary mediator for ensuring the integrity of the gastric mucosa, and COX-2 is primarily involved in inflammation. COX-2 selective NSAIDs should provide anti-inflammatory relief without compromising the gastric mucosa [2].

Pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-6, IL-1β, and IL-8, need to be highlighted due to their significant participation in increasing the inflammatory response.

Structural and functional analyses of selective COX-2 inhibitors in the COX active site cavity could allow the introduction of lead structures with more excellent selectivity and potency against inflammation with fewer adverse effects.

This review focuses on the biological activity of newly discovered synthetic COX-2 inhibitors, COX-2 hybrid inhibitors, COX-2/lipoxygenase dual hybrid inhibitors, and COX-2/epoxide hydrolase soluble hybrid inhibitors which are based primarily on the active motifs of related drugs approved by the US Food and Drug Administration [2].

NSAIDs are acidic compounds with variable hydrophobicity. As analgesics, NSAIDs are effective against low or moderate-intensity pain. Antipyretics reduce body temperature in febrile states [3, 4]. Still, their main activity and clinical application are anti-inflammatory agents in treating musculoskeletal conditions such as rheumatoid arthritis and osteoarthritis [4, 5].

Examples of non-steroidal anti-inflammatory drugs (NSAIDs) and pharmaceutical classes are shown in Table 1.

Table 1 Drug class of non-steroidal anti-inflammatory drugs (NSAIDs) [3]

Pharmacological class	**Name**
Acetic acid derivatives	Indomethacin
	Aceclofenac
	Diclofenac combinations
	Sulindac

	Diclofenac
Butylpyrazolidine	Phenylbutazone
Coxibs	Valdecoxib
	Rofecoxib
	Celecoxib
	Etoricoxib
Fenamat	Tolfenamic
Oxycam	Tenoxycam
	Meloxycam
	Pyroxicam
Propionic acid derivatives	Dexibuprofen
	Thiaprofenic acid
	Flurbiprofen
	Ketoprofen
	Naproxen
	Ibuprofen
Other	Nabumetone

1.3. NSAID production and pharmaceutical market

Musculoskeletal drugs represented the largest pharmaceutical market worldwide, with 14%. of the total in 2017. The second, third, and fourth largest markets were cardiovascular, oncology, and anti-infective drugs.

The fifth-largest market was drugged to treat metabolic disorders such as diabetes, thyroid, and pituitary diseases will be the fastest growing segment of the global pharmaceutical market by 2021. This segment will increase by 9% per year in the future, after a recent growth of 11.6%.

The DrugBank 2019 database (version 5.1.3, released on 2 April 2019) contained 13,336 drugs, 10,256 were small molecule drugs, and 1670 were biotech, while 3732 were approved, 2593 were small molecule approved, 130 were nutraceuticals, 6304 were experimental, 205 were illicit, and 256 were withdrawn drugs.

In addition to the pharmaceutical market, global pharmaceutical consumption has also been growing, partly driven by the increasing need for medicines to treat age-related and chronic diseases and changes in clinical practice [4, 5].

It is estimated that non-steroidal anti-inflammatory drugs (NSAIDs), one of the most important groups of drugs, are produced in quantities of several thousand tonnes per year [6]. NSAIDs are commonly used in analgesic, antipyretic and anti-inflammatory treatments and in preventing myocardial infarction [7]. For example, paracetamol consumption worldwide has increased. It is ranked among the top three prescription drugs in England and the top 200 prescription drugs in the US [8]. In the UK, about 3.2 × 109 tablets of paracetamol are consumed each year; in the US, about 3.6 × 109, an average of 55 tablets/person [9]. In the Nordic countries, paracetamol consumption exceeds 20 g/person/year [10].

Diclofenac is included in the emergency medicine list (EML) of 74 countries. The exact annual consumption of diclofenac in North America is not available, even though in North America, DCF's market share in the drug market is growing. In the US, DCF contributes to about 5-6% of the total NSAID market, while in Canada, 17% of the NSAID market consumed is DCF. Consistent with the current trend, DCF consumption will continue to increase in North America as lifestyle-related diseases, such as arthritis and heart disease, become common. Also, the aging population will need medications such as painkillers [11].

Annual data on consumption or prescription of diclofenac are available for some countries. According to consumption estimation models, in Australia, it has been estimated that 4 tonnes of DCF are used annually [12]. In Europe, the largest user of DCF is Germany, with 86 tonnes of DCF in 2001 [13]. For the rest of the European countries (at 2001 level), consumption is England 26.13 tonnes per year, Austria 6,14 tonnes per year [24] and France 16 tonnes per year [14-18].

Total consumption of DCF across Europe was estimated at 179.8 tonnes per year [19]. For most Asian and African countries, DCF consumption data are unavailable due to the lack of consumption surveys and sales inventory. Given the frequent reports of toxicological effects observed in these countries on vultures, it can be assumed that consumption can be colossal [11].

Recent studies based on IMS Health data (serving 82% of the world's population) from 86 countries estimated that an average of 1443 ± 58 tonnes of DCF is currently consumed worldwide [20].

CHAPTER 2

2. Drug pollution of water and soil

In terms of sources of pollution, the main source of drug pollution is the patient himself.

After acting in the body, drugs taken by the patient are excreted together with urine or feces, without being metabolized or as active metabolites entering the sewage.

Figure 7 shows the possible sources and pathways of drug residues in soil and water.

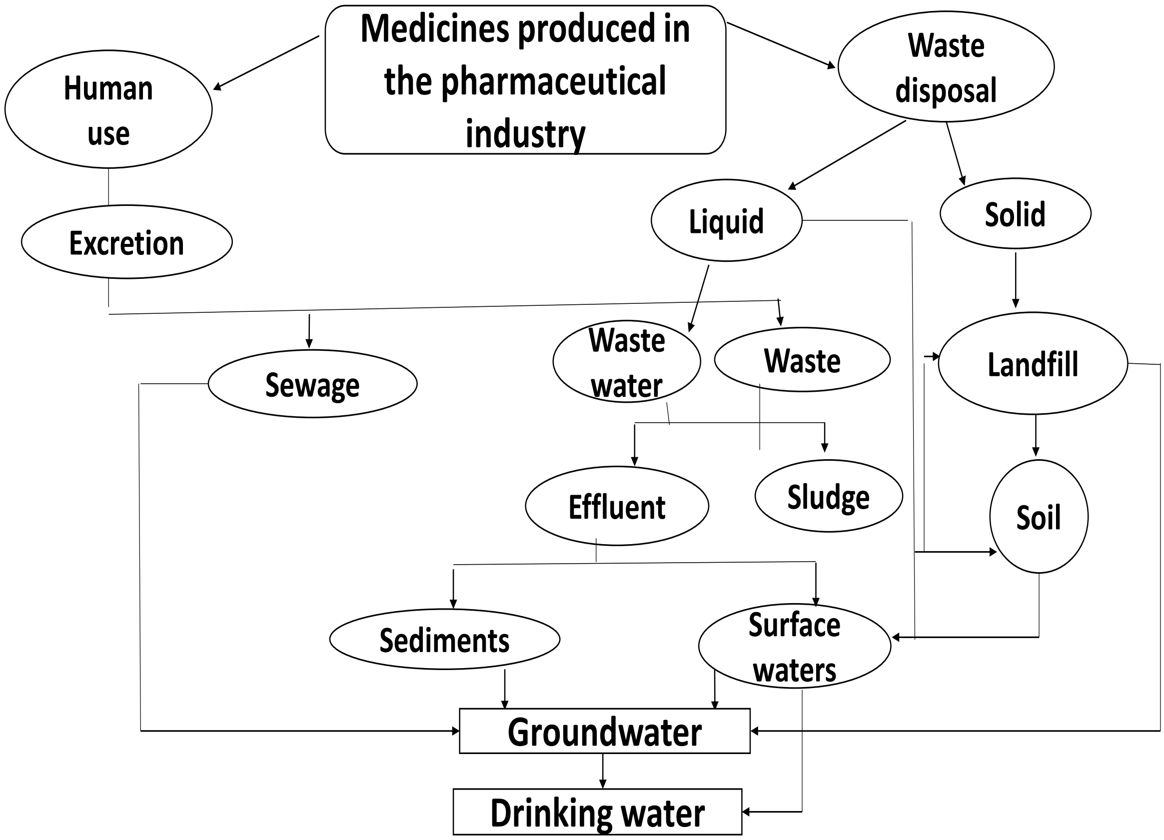


Figure 7. Possible sources and pathways of drug residues in soil and water.

EXPERIMENTAL PART

CHAPTER 5

5. Influence of paracetamol on *Phaseolus vulgaris* L. plants

This chapter examined the influence of paracetamol on bean (Phaseolus vulgaris L.) plants. On the one hand, different concentrations of paracetamol (1 g L^{-1}, 2 g L^{-1}, 3 g L^{-1} and 4 g L^{-1}) were used on a single bean variety. Also, at the maximum concentration (4 g L^{-1}), it was studied how several bean varieties respond to the presence of paracetamol. Different varieties of *Phaseolus vulgaris* L. were used (Minidor (GBBR, Bekescsaba Hungary), Fideluță (Mefim Agro Bekescsaba Hungary), Odir (GBBR, Bekescsaba Hungary) and Ecaterina (Agrosel, Câmpia Turzii, Romania)) to determine how paracetamol influences the growth and development of bean plants.

5.1. Study of the Influence of paracetamol concentration on plants

5.1.1. The Influence of paracetamol on photosynthesis parameters

Net assimilation rates and stomatal conductances to water vapor are not significantly different from the control for plants treated with 1 g L^{-1} paracetamol. Assimilation rate decreases by more than 20% in plants treated with 2 g L^{-1} paracetamol (Figure 17).

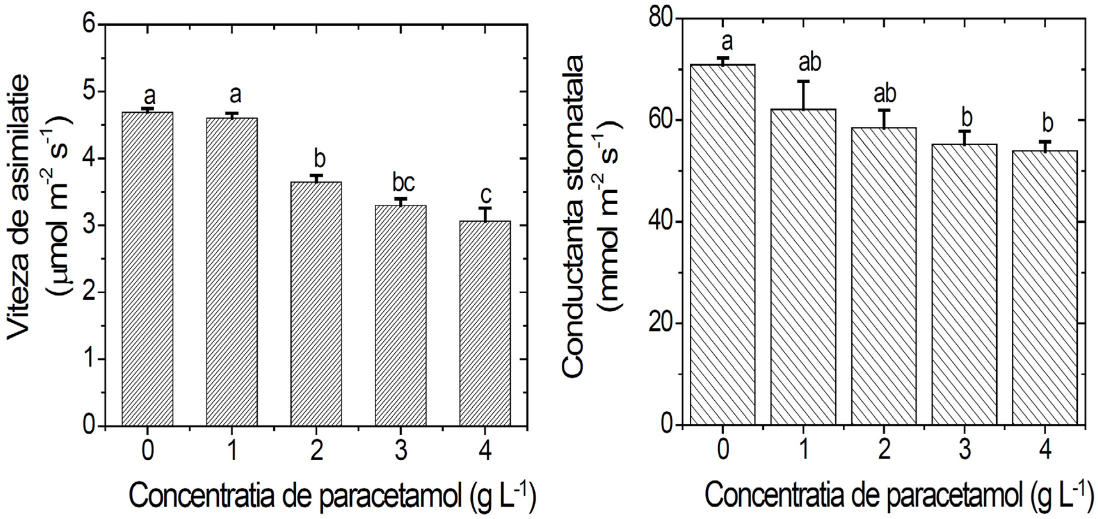


Figure 17. Changes in assimilation rate and stomatal conductance for Phaseolus vulgaris L. cv 'Odir' treated with paracetamol.

The same trend was observed for stomatal conductance, but in this case, there were no significant differences between plants treated with a concentration higher than 2 g L^{-1} paracetamol. Such behavior of assimilation rate and stomatal conductance indicated decreased potential photosynthetic activity of the plant in all treatments compared to control plants. Our results agree with [181], which demonstrated a decrease in chlorophyll fluorescence ratio for paracetamol-treated plants.

5.2.1. influence of paracetamol on photosynthesis parameters in different bean varieties

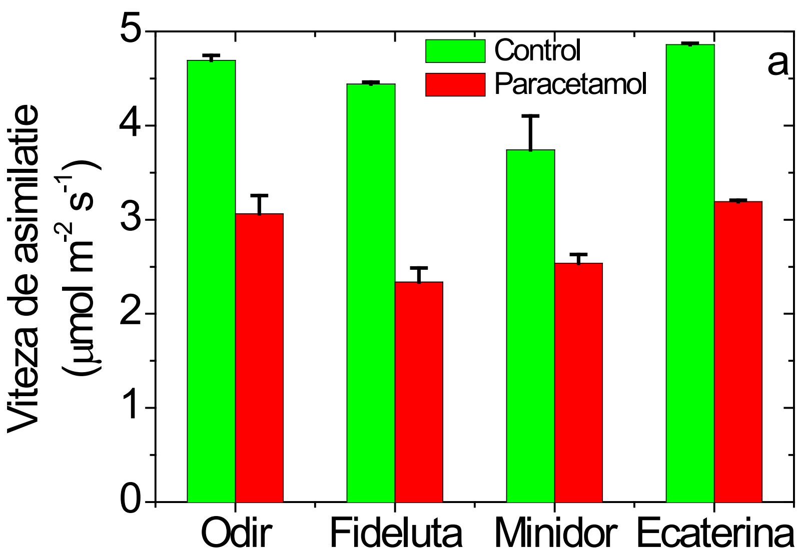
Photosynthesis is strongly influenced by drought, cold, salt, heat, oxidative stress, heavy metal toxicity, and other stressors [208].

Stress conditions destroy chloroplast ultrastructure and lead to a decrease in the amount of chlorophyll, resulting in lower photosynthetic activity [209].

From (Figure 23 a)) it can be seen that the rate of carbon dioxide assimilation for plants treated with paracetamol decreases but also that there are significant differences between bean varieties, Fideluță and Minidor being the most affected by the presence of paracetamol. These differences can be explained by the different susceptibility of these varieties to stress, as some varieties are known to be more resistant and others less resistant.

The amount of stomatal conductance governs plant water quantity and photosynthesis, which is severely affected by various stress factors [210].

Stress has also been shown to negatively influence transpiration rate [211] and affect CO_2 fixation by reducing the synthesis and causing degradation of enzymes involved in the CO_2 assimilation process.



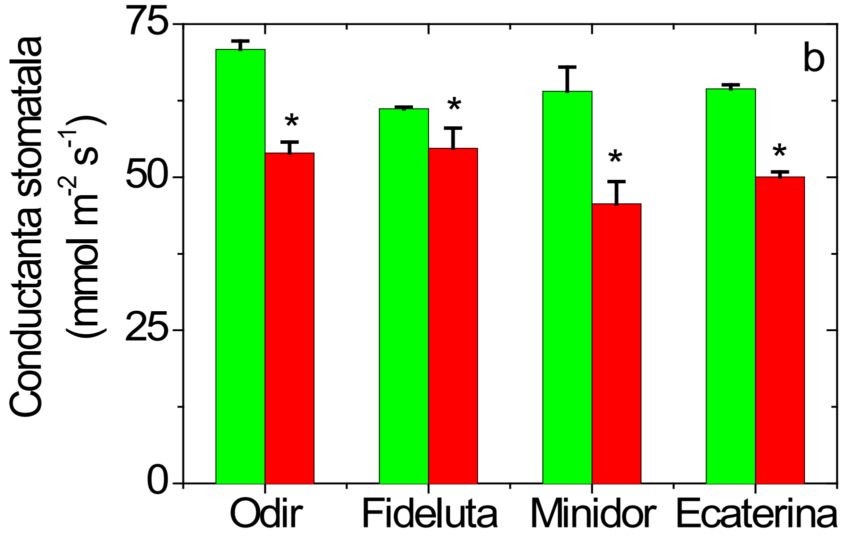
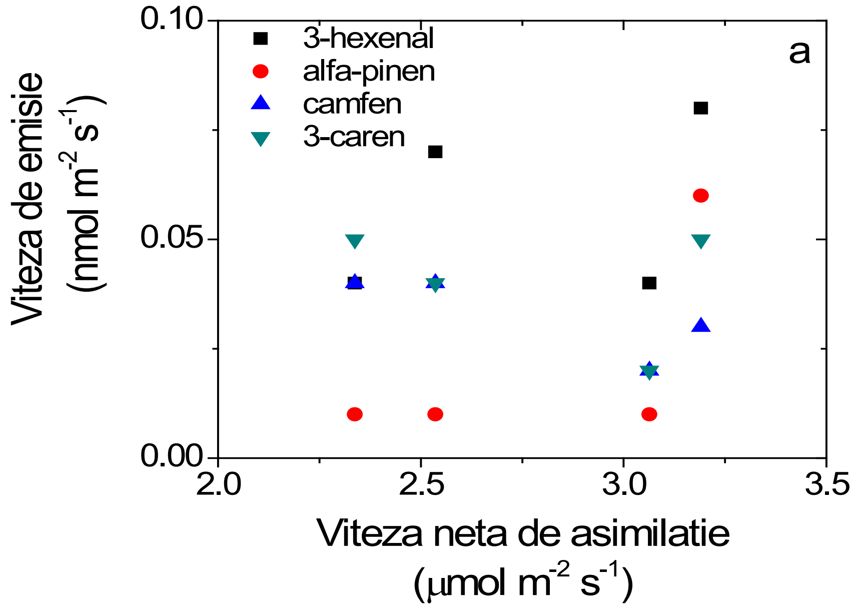


Figure 23. Variation in assimilation rate (a) and stomatal conductance (b) for different bean varieties treated with 4 g L-1 paracetamol

5.2.4. Correlations between different parameters

In order to determine how high paracetamol concentration influences various plant physiological parameters, the correlation between photosynthesis parameters and emission of volatile organic compounds was attempted.



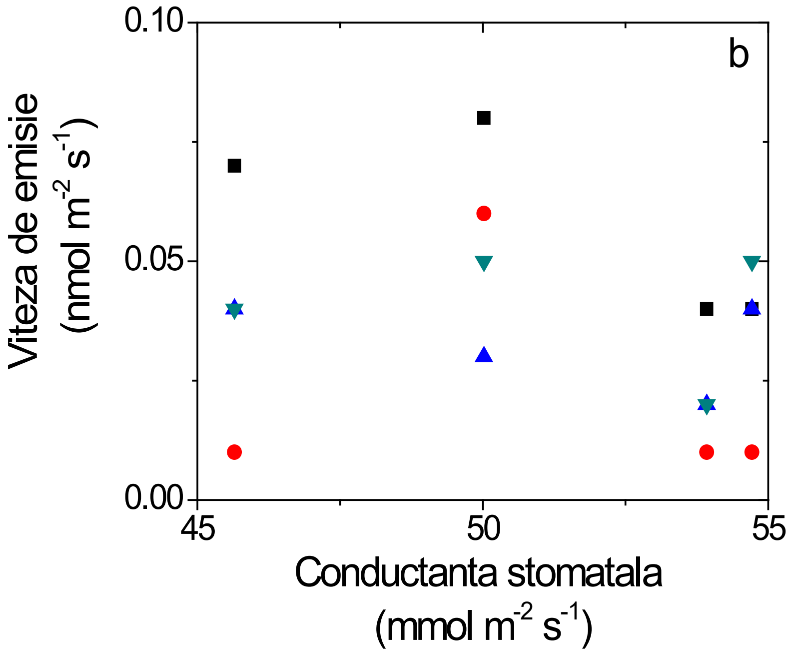


Figure 27. Correlation between terpene emission rate and assimilation rate (a) and stomatal conductance (b).

It is observed that there is no correlation between terpene emission rates and photosynthesis parameters which means that the way paracetamol intervenes in metabolic cycles is different.

5.3. Conclusions

Residual paracetamol in the soil can become an important plant stressor even at g L^{-1} concentrations.

Plants grown in paracetamol-contaminated soil show decreased photosynthetic parameters and secondary metabolism compounds at lower concentrations than control plants affecting their antioxidant capacity.

It has been shown that different varieties of *P. vulgaris* react differently to paracetamol-induced stress, especially in photosynthesis parameters.

Linear correlations between net assimilation rate and chlorophyll *a* have been shown for stressed plants.

CHAPTER 6

6. Influence of diclofenac on plants of *Phaseolus vulgaris* L.

This chapter investigated the influence of various concentrations of diclofenac 0,1; 0,2; 0,3 and 0,4 g L-1 on bean (*Phaseolus vulgaris* L.) plants. For the experiments, bean plants (*Phaseolus vulgaris* L.) var. Ecaterina (Agrosel, Câmpia Turzii) is a variety of beans with large leaves and fleshy beans. The growing method and conditions were as described in Chapter 4.

6.2. Influence of diclofenac on photosynthesis parameters

Photosynthesis parameters of bean plants grown in the presence of diclofenac at various concentrations were determined. The results obtained are shown in (Figure 32).

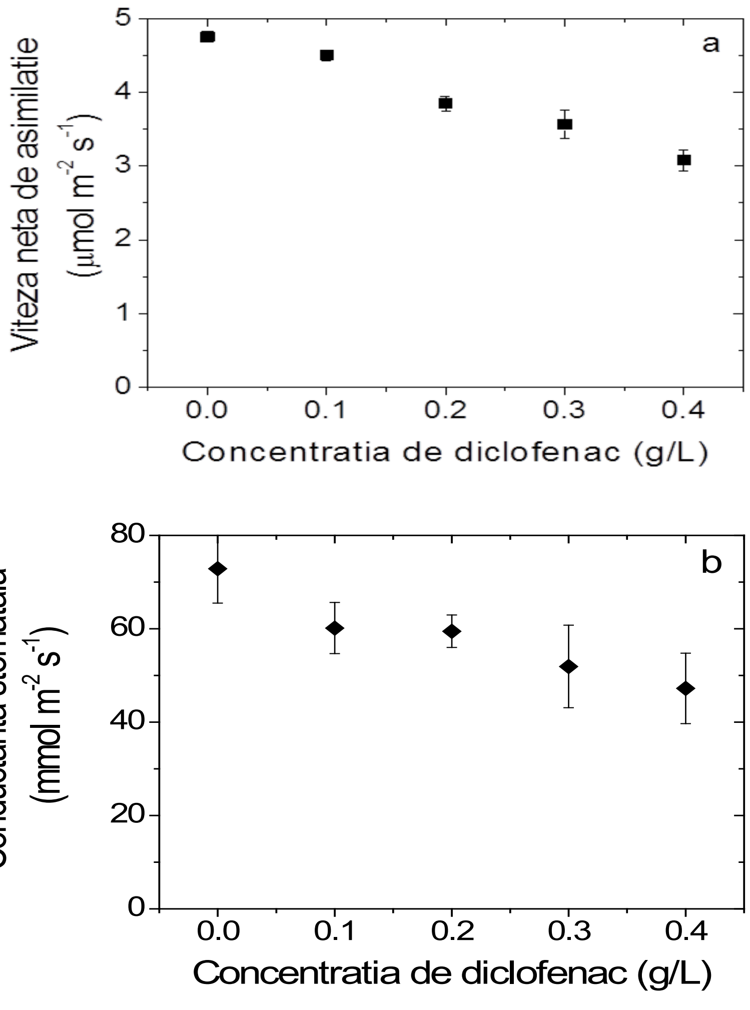


Figure 32. Dependence of photosynthesis parameters on diclofenac concentration

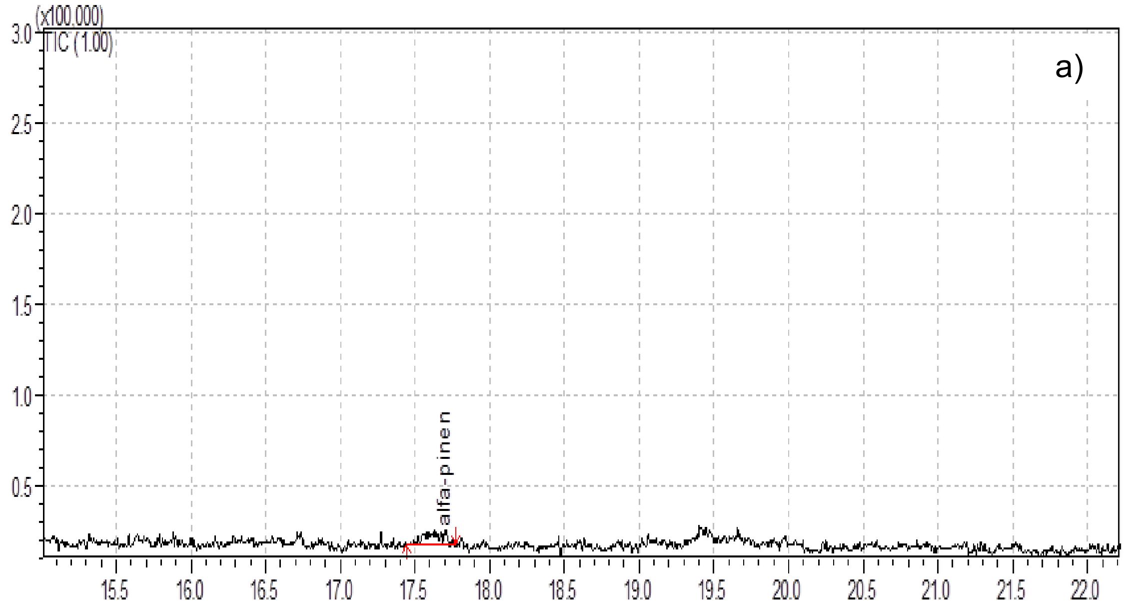
A linear decrease in the values of both parameters is observed with increasing diclofenac concentration. The downward trend is more pronounced for the net assimilation rate.

The main steps of photosynthesis are reactions in the presence of light in the PSI and PSII photosystems (including, in the latter case, the Hill reaction) and CO_2 assimilation in the Calvin cycle. In addition to structural damage to PSII and PSI photosystems, DCF disrupts electron flow between photosystems. Water loss occurs not only via the stomata but also through the cuticular pathway.

Similar to the stomatal opening, the cuticular pathway is affected by the growth environment and the presence of stressors.

6.3. Influence of diclofenac on VOC emission rate

A typical chromatogram for volatile organic compounds emitted by plants is shown in (Figure 33 b)).



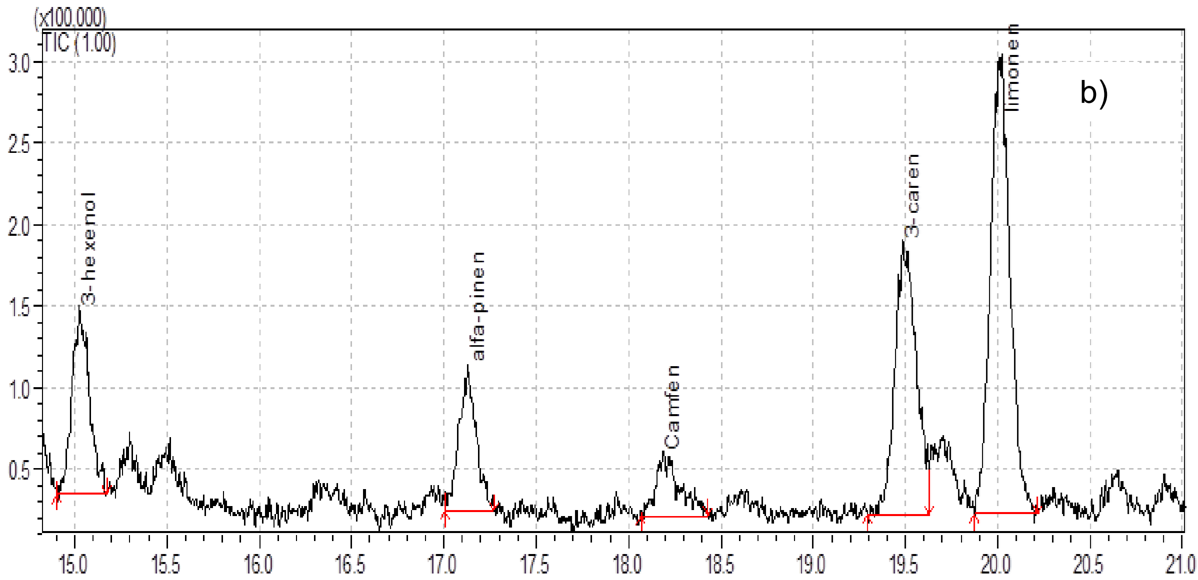


Figure 33. Typical chromatograms for emission of volatile organic compounds from leaves for control plants (a) and 4 g L^{-1} diclofenac-treated plants (b), respectively.

Based on the mass spectra, one compound from the class of C_6 unsaturated aldehydes and four monoterpenes were identified, although the concentrations of the emitted compounds are deficient.

6.6. Conclusions

Diclofenac has been shown to influence the growth of bean plants at concentrations usually found in soil.

Bean leaves' photosynthesis parameters (net assimilation rate and stomatal conductance) are low for diclofenac-treated plants.

Volatile organic compounds emitted by plants (especially 3-hexenol) depend on the concentration of diclofenac.

The concentration of chlorophylls and carotenoids in leaves decreases with increasing stressor concentration.

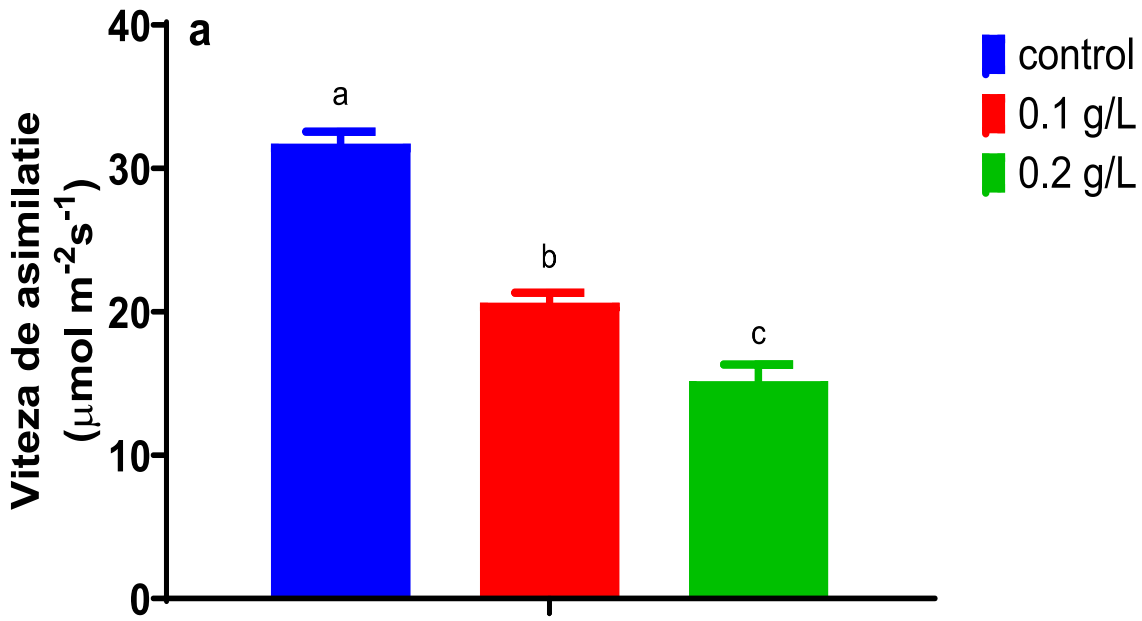
CHAPTER 7

7. Influence of algocalmin on plants of *Phaseolus vulgaris* L.

To study the influence of algocalmin on plants, the bean variety Ecaterina was treated with 0,1 and 0,2 g L^{-1} metamizole sodium in water. The plants were grown for three weeks, and determinations were performed as described in Chapter 4.

7.1. Influence of metamizole on photosynthesis parameters

The influence of the two concentrations of metamizole on the photosynthesis parameters (assimilation rate and stomatal conductance) is shown in (Figure 40). A drastic decrease of both parameters is observed, reaching a concentration of 0,2 g L^{-1} at about 50% compared to control plants. Thus, due to the toxicity of this drug, the functioning of the photosynthetic electron transport chain from PSI (photosystem I) to PSII, carbon assimilation, and photorespiration were impaired. The high concentration and toxicity of metamizole irreversibly damaged leaf cells. This may be due to the accumulation of NSAIDs over time or their metabolic conversion into bioactive compounds.



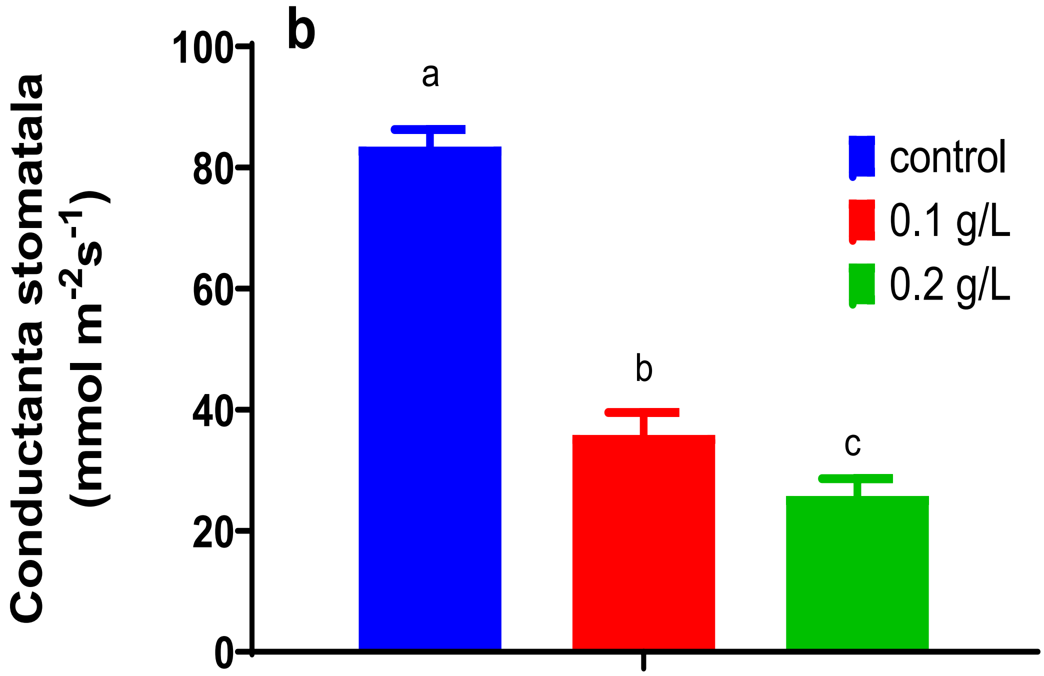


Figure 40. Dependence of photosynthesis parameters on metamizole concentration

Such behavior, with an extremely strong decrease in stomatal conductance, suggests a disturbance of the Ca balance that alters the photochemical phase and affects photosystem II (PSII), which reduces the stability and aggregation of chlorophyll molecules in the antenna complex.

7.6. Conclusions

Exposure to algocalmin reduced chlorophylls, carotenoids, polyphenols, and flavonoids in a dose-dependent manner.

Photosynthesis parameters (net assimilation rate and stomatal conductance) decrease drastically for plants treated with algocalmin.

Due to the toxicity of algocalmin and probably its high solubility in water, plants grown on soil containing this compound may suffer changes in their metabolic cycles.

CHAPTER 8

8. Influence of ketoprofen on plants of *Phaseolus vulgaris* L.

This chapter investigated the influence of ketoprofen at a concentration of 4 g L-1 on different varieties of beans (Phaseolus vulgaris L.). Three varieties of beans were used as follows: Minidor, (GBBR, Bekescsaba Hungary), Fideluță, (Mefim Agro Bekescsaba Hungary), and Odir (GBBR, Bekescsaba Hungary) (Figure 47).

Figure 47. Bean plants used in the experiment

8.2. Influence of ketoprofen on photosynthesis parameters of bean leaves

From (Figure 50), it can be seen that the uptake rate for plants treated with ketoprofen decreases but also that there are significant differences between bean varieties, Fideluță and Minidor being the most affected by the presence of ketoprofen.

8.3. Influence of ketoprofen on the emission of volatile organic compounds from bean leaves

In the mixture of compounds emitted from ketoprofen-treated bean leaves, the presence of a volatile green leaf volatiles (3-hexenol) and three monoterpenes (-pinene, camphene, and 3 carene) was determined (Figure 51 shows a typical chromatogram).

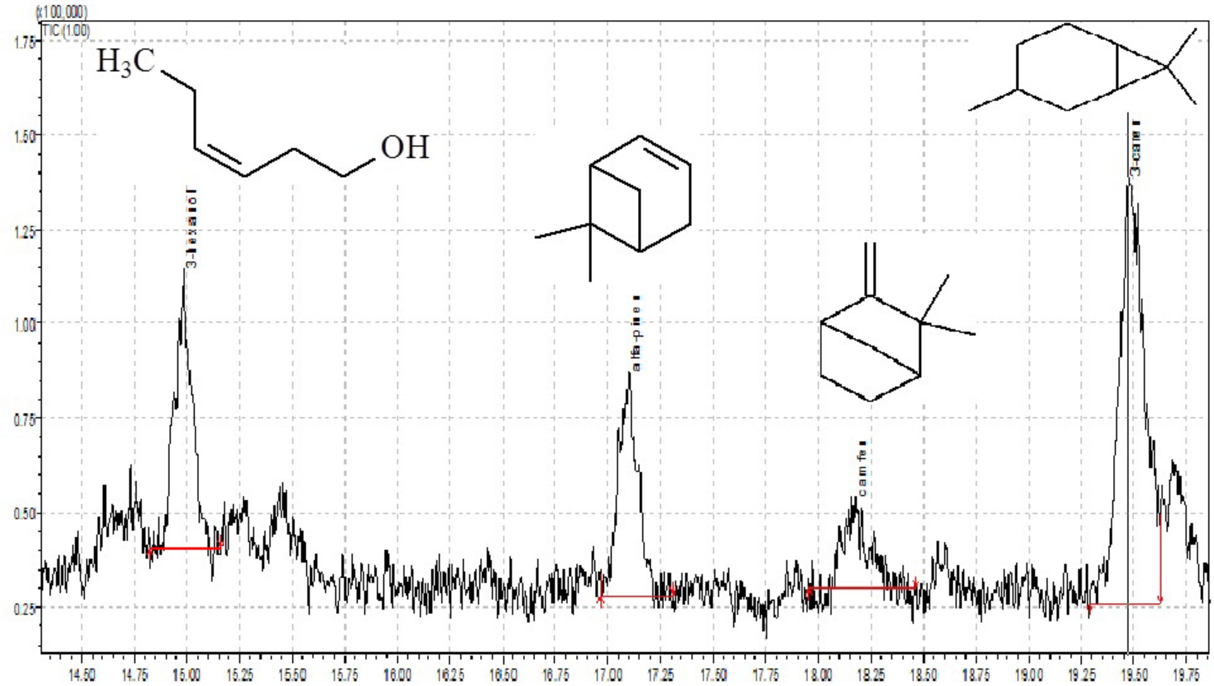


Figure 51. Typical chromatogram for emission of volatile organic compounds from leaves treated with 4 g L^{-1} ketoprofen

8.5. Conclusions

The presence of ketoprofen at a relatively high concentration in soil has been shown to influence plant growth and development by affecting photosynthesis parameters.

It has been shown that different bean varieties do not react differently to ketoprofen-induced stress.

Chlorophylls and β-carotene are affected for all varieties at the concentration of ketoprofen taken as working with no differences between bean varieties.

CHAPTER 9

9. Influence of non-steroidal anti-inflammatory drugs on plants of the *Fabaceae* family

Plant behavior has been reported to be dose-dependent [21, 22].

The concentration of NSAIDs used in this study was lower than the maximum values determined for drugs found in the environment [112, 230, 231].

Since all NSAIDs have the same adverse effects on the assimilation rate, all these drugs may have the potential for phytotoxicity. In addition, the decreased activity of PSII response centers is probably related to the suppression of photosynthesis.

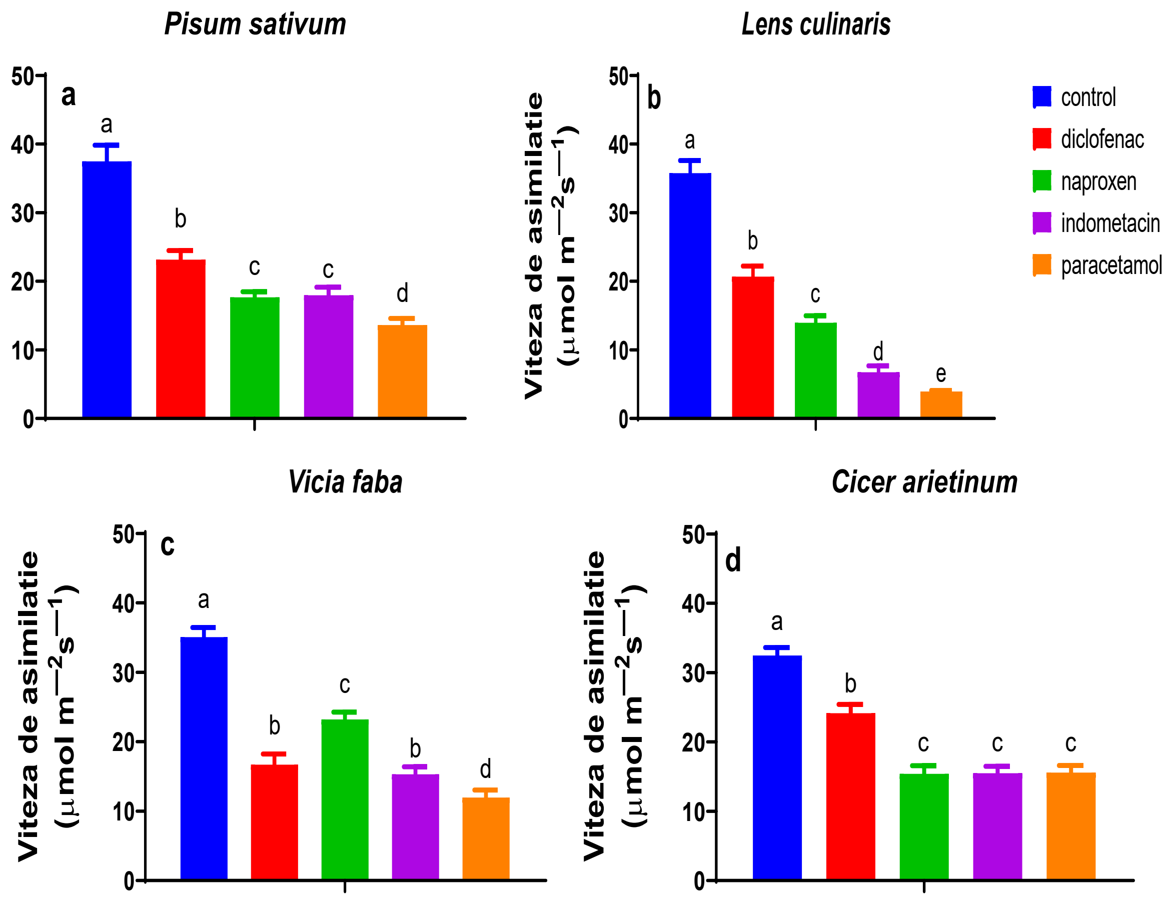


Figure 54. The assimilation rate from *Pisum sativum* (a), *Lens culinaris* (b), *Vicia faba* (c), and *Cicer arietinum* (d).

9.6. Correlations between parameters

From the principal component analysis (PCA) plot with only photosynthetic parameters and photosynthetic pigments (Figure 63), the *Cicer arietinum* plants were the most influenced by the presence of NSAIDs. In contrast, the *Pisum sativum* plants were less disturbed. It has already been shown that chickpea plants are sensitive to soil salinity [23] and temperature [24], and the present study demonstrates, once again, its sensitivity to abiotic stresses.

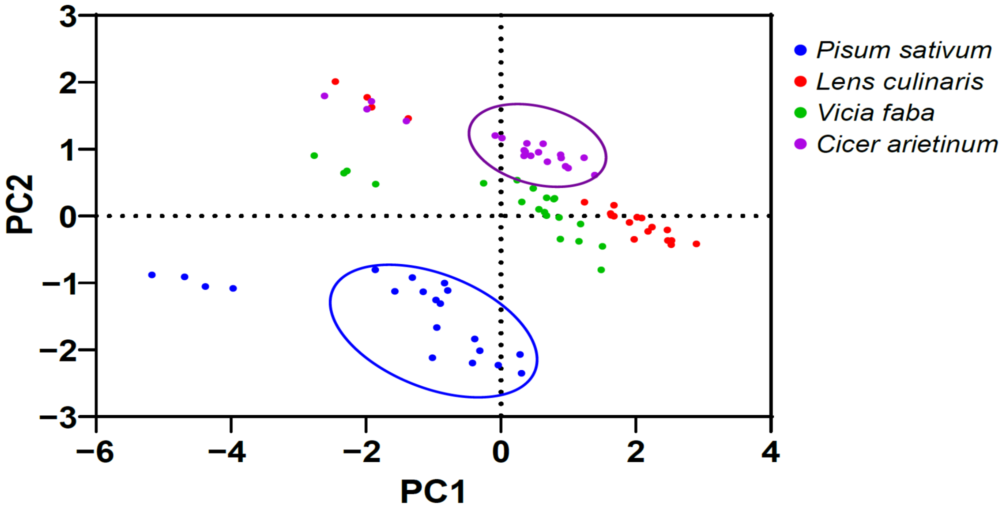


Figure 63. The principal component analysis (PCA) plot with photosynthesis parameters and pigments PCA 1 and 2 explain 84.5% of the variation (61.9% and 22.6%, respectively) (raw data in Appendix 1).

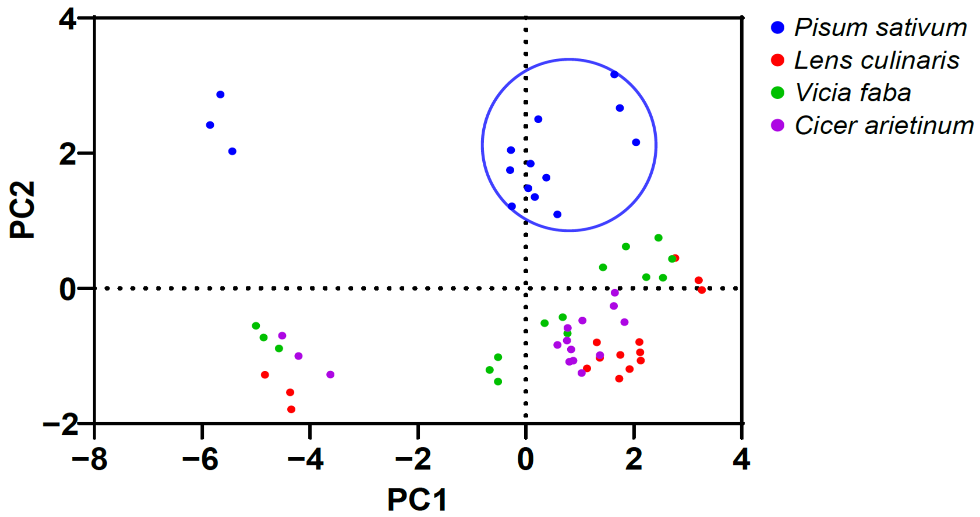


Figure 64. The principal component analysis (PCA) diagram (raw data in Appendix 1).

From the PCA plot with all available data (Figure 64), the clustering of control plants could be seen compared with the treated plants. The *Pisum sativum* plants express high sensitivity to NSAIDs, but there is no clear pattern for the other species.

When plotting the PCA for drug variation, it is observed that although the control plants show clustering, no clear dependence can be detected for any of the drugs considered.

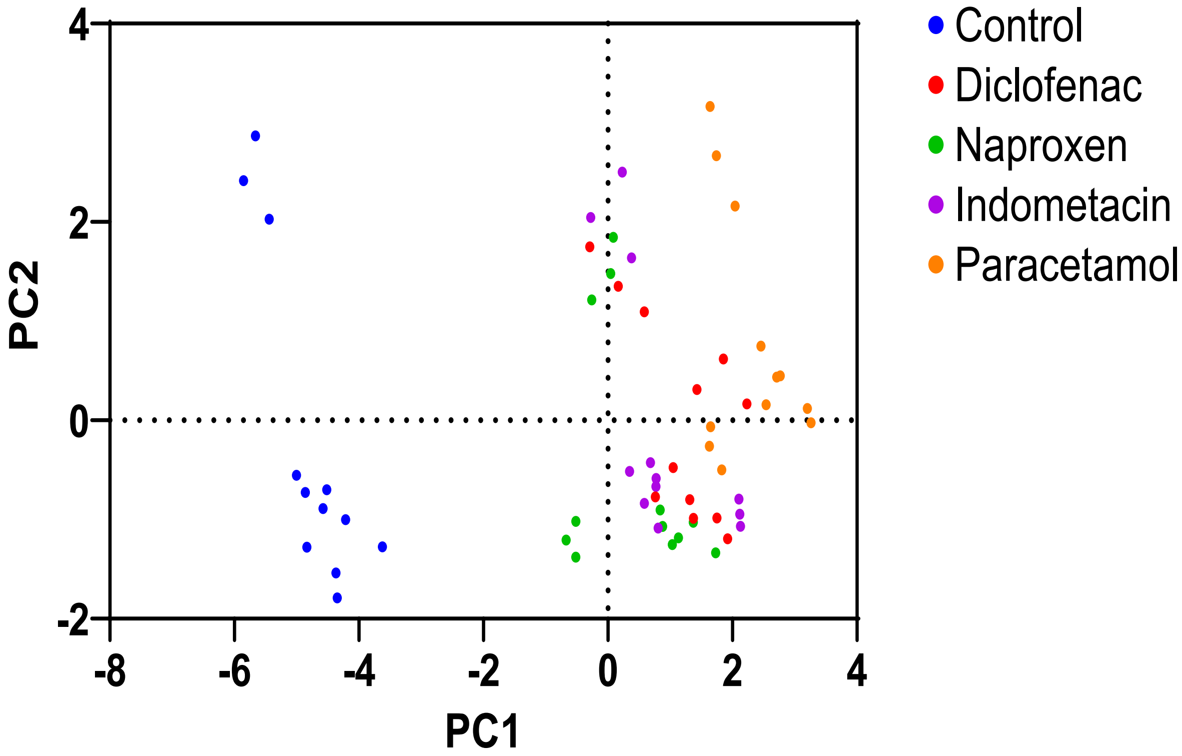


Figure 65. The principal component analysis (PCA) diagram (raw data in Appendix 1).

On the other hand, from the graph shown in (Figure 66), it can be seen that the emission of volatile organic compounds is influenced in the same way regardless of the compound studied over time. The parameters of photosynthesis and β-carotene are affected similarly, clearly explaining the link between them.

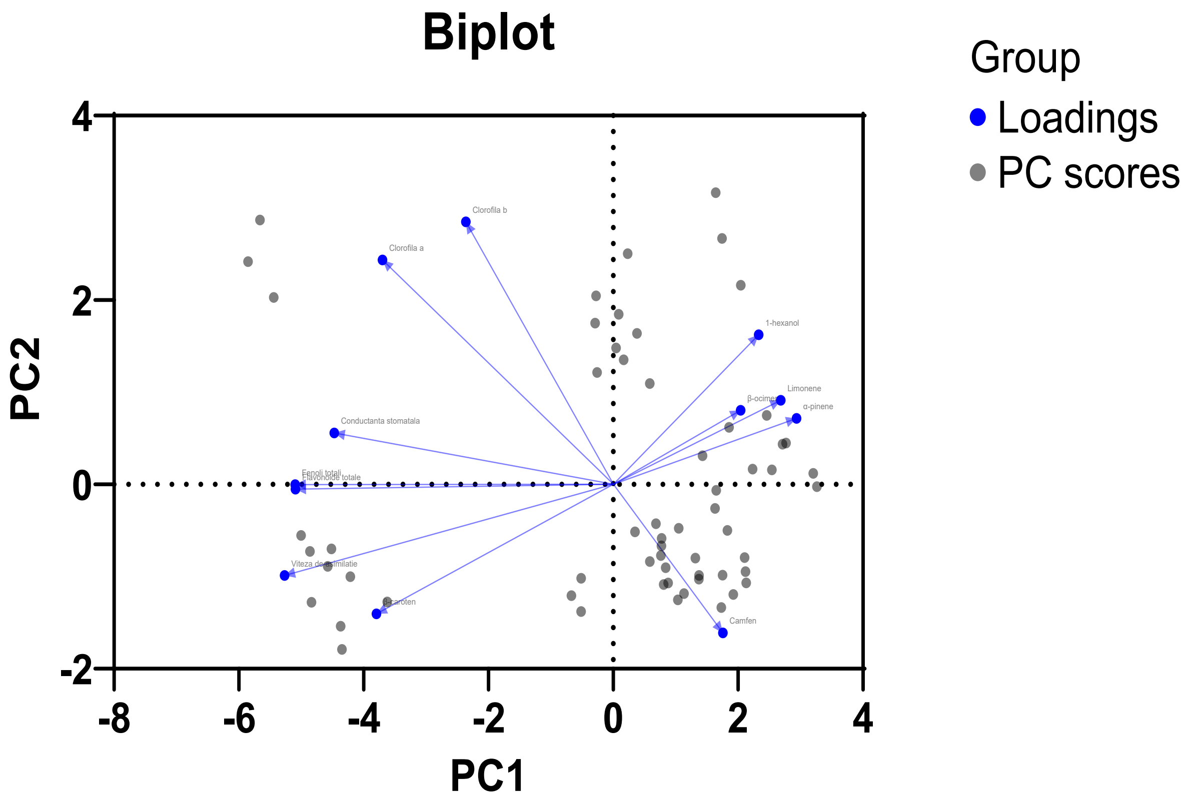


Figure 66. The principal component analysis (PCA) diagram

9.7. Conclusions

This research shows the impact of four significant organic contaminants (diclofenac, indomethacin, naproxen, and paracetamol) on the composition and ultrastructure of food-producing plants.

Exposure to photosynthetic parameters, chlorophyll, carotenoids, polyphenols, and flavonoids was reduced due to NSAIDs.

In contrast, the emission of different terpenes and green leaf volatiles involved in secondary aerosol formation was enhanced even for plants not known to be volatile compound emitters.

GENERAL CONCLUSIONS

This work studied how non-steroidal anti-inflammatory drugs (NSAIDs) influence photosynthesis parameters, chlorophyll, and carotenoid pigments, and secondary metabolites in plants of the *Fabaceae* family.

The effect of paracetamol in the environment was tested on *Phaseolus vulgaris* L. It was shown that different bean varieties react similarly to the presence of paracetamol, although some are less resistant to micropollutants. Photosynthesis parameters (assimilation rate and stomatal conductance) are less dependent on bean variety than on the emission of volatile organic compounds. On the other hand, the influence of various paracetamol concentrations (at soil or groundwater levels) on a Romanian bean variety (Ecaterina, Agrosel, Campia-Turzii) was determined. It has been shown that assimilation rate and stomatal conductance decrease for plants treated with more than 1 g L^{-1} paracetamol. Also, the emission rate of volatile organic compounds increases directly proportional to the concentration of the stressor. The same linear dependence (but in this case, decreasing) was observed for the content of chlorophyll and carotenoid pigments (especially chlorophyll *a*).

The effect of diclofenac on *Phaseolus vulgaris* L. plants has been studied for various drug concentrations showing that diclofenac decreases germinated seed percentage, assimilation rate, stomatal conductance, and chlorophyll and carotenoid pigments even at extremely low concentrations (0,1 g L^{-1}). Again, a linear dependence between drug concentration and the parameters analyzed is observed.

It has been shown that exposure of *Phaseolus vulgaris* L. plants to metamizole (algocalmin) resulted in drastic decreases in photosynthesis parameters (net assimilation rate and stomatal conductance). A reduction of chlorophylls, carotenoids, polyphenols, and flavonoids was also dose-dependent depending on metamizole concentration.

The influence of ketoprofen on various varieties of *Phaseolus vulgaris* L. was studied for a concentration commonly found in soil. It was observed that photosynthesis parameters, chlorophyll *a* and chlorophyll *b* concentration, β-carotene from leaf, decrease. At the same time, VOC emission rate increases significantly for all varieties compared to control plants but no significant differences are observed between the different varieties studied.

Taking into account the observations in the previous chapters, a concentration of 0,5 g L^{-1} was chosen. Four species of the Fabaceae family (pea, lentil, chickpea, and cowpea) were treated with five NSAIDs (paracetamol, naproxen, indomethacin, ketoprofen and diclofenac). In all cases, major influences were observed on photosynthesis parameters, chlorophyll concentrations and β-carotene, and total phenolic and flavonoid content.

Principal component analysis showed that pea plants are less resistant to drug-induced stress. On the other hand, it has been shown that it is impossible to rank the toxicity of the NSAIDs used for the plants under study.

In terms of the original contributions of the thesis, the following stand out:

• Determination of the emission of volatile organic compounds from plants treated with various NSAIDs. These VOC emissions in the presence of stress may have implications for photochemical reactions in the atmosphere and the formation of secondary aerosols;

• The Influence of metamizole (algocalmin) on plants has been studied for the first time;

• It was determined dependent, stressor-response dose in the plant, showing that there is a linear dependence;

• It has been shown that different varieties of beans do not respond differently to NSAID stress;

• Principal component analysis indicates that the toxicity of the various NSAIDs is similar, at least for plants of the *Fabaceae* family.

The insights that emerge from this thesis are:

• To better understand the potential impact of pharmaceuticals and their mixtures on plants (either on their sustainability or on productivity and food security), further phytotoxicological studies should be considered, particularly chronicity tests.

• Because of the strong influence of environmental conditions, plant species, drug concentrations, mixtures, and exposure times, as well as other biological, physical, and mechanical stressors that may be involved, in situ observations are required.

• Since these drugs are transformed in the human and animal body, and a wide variety of metabolites are excreted, tests are performed either with the resulting metabolites or with the excreted substances.

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