# Biochemical and Physiological Effects of Some Organic and Inorganic Chemical Agents in *Capsicum spp.*

**ELENA TODIRASCU CIORNEA[1]*, ELENA GROSU[2], DIANA ELENA BUCUR[2], ANDREI LOBIUC[1]**

[1] Alexandru Ioan Cuza University Iasi, Faculty of Biology, 20A Carol I Blvd., 700505, Iasi, Romania

[2] Biological Researches Institute Stejarul, 6 Alexandru cel Bun Str., 610004, Piatra Neamt, Romania

*Cultivation of vegetables is expected to increase in order to meet the demands of the expanding populations of the globe. Meanwhile, anthropic activities increase the concentrations of various chemical compounds in aquatic and terrestrial habitats. For productivity and food safety reasons, assessments of effects of pesticides and metals on crops should be performed. In the current paper, the presence of some heavy metals and a pesticide compound in the substrate altered the levels of some Krebs cycle enzymes activities in pepper plants cultivated in controlled conditions. The photosynthetic apparatus of the same plants appeared relatively unaffected, while the potential/actual soil dehydrogenases ratios were increased in all treatments.*

*Keywords: pepper, chemical treatments, enzymes, pigments*

During the past years, the development of industry and modern practices used in agriculture led to the widespread of soil contamination with heavy metals and compounds from pesticides [1]. This represents a concern because legumes cultivation may lead to the uptake and storage of metals in tissues, causing toxic effects in plants, but in the same time, it may represent a contamination risk of alimentary products [2]. In addition, it is known the fact that the pesticides that contain dinitrophenol might damage the environment [3,4**]**, their action being based mainly on blocking the oxidative phosphorylation reactions and on the inhibition of ATP formation starting with ADP [5]. The absorption capacity of heavy metals from soil is different depending on the type of plant [6]. Thus, the legumes present a low capacity to absorb heavy metals from soil the root vegetables have a moderate tendency to assimilate trace elements, while the leafy vegetables accumulate the largest amount of metals [7].

The high interest in studying species from the *Capsicum* genus is due to its varied content of nutritional compounds and pharmacologically active metabolites [8]. Thereby, while the *Capsicum* fruits are characterized by high concentration of ascorbic acid, carotenoids and calcium [9], it was reported that the capsaicinoids encountered in the leafs and the stems of pepper plants may have a significant antibiotic, carminative, aphrodisiac, antiasthmatic, and antitumor effect [10].

The current paper aims to assess the biochemical and physiological response of *Capsicum* plants in the presence of varied types of organic and inorganic substances in the substrate, compounds that may act as potential pollutants in the growth environment.

## Experimental part

### *Plant material*

The researches were performed on two pepper cultivars, namely *Capsicum annuum* cv. Macska sarga, respectively *Capsicum baccatum* var. *pendulum* cv. Aji Amarillo variety, cultivated in laboratory conditions. The seeds were germinated on jiffy peat pellets with a diameter of 33 mm, moistened with 40 mL of distilled water, and then distributed in alveolar trays for seedlings, each one having 10 square wells with 6 cm sides. After germination the seedlings were watered each two days with 10 mL of

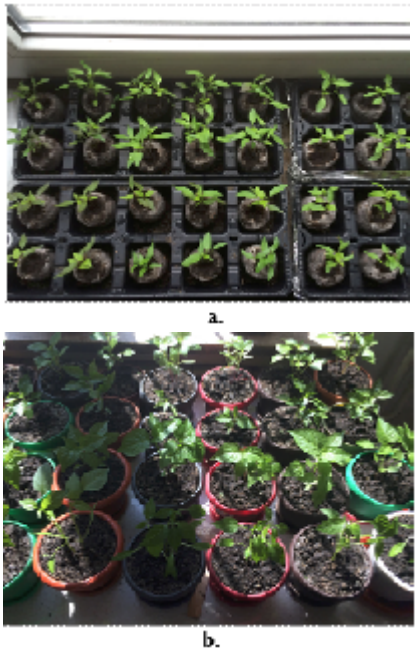


Fig.1. Pepper seedlings

MilliQ distilled water, for a month (fig. 1a). Then, the seedlings were transplanted in pots with a diameter of 13.5 cm (Fig. 1b) and treatments were applied with a variety of organic (dinitrophenol 0.001 M -substance that enters into the composi-tion of different pesticides, herbicides, fungicides, ovicides, acaricides etc., and that is frequently used in the modern agricultural practices; and Atonik -a growth, rooting and fructification regulator that contains 0.1% nitroguiacol sodium, 0.2% o-nitrophenolat sodium and 0.3% p-nitrophenolat sodium) and inorganic agents ($FeSO_4$, $(CH_3COO)_2Pb$, $NiCl_2$, $SnCl_2$ - the solutions used being prepared in such way that the final concentration of the metal was 0.001 M). The harvesting and analyses were performed at 60 days after the beginning of the treatment, for each three replications per variant, the results being expressed as average ± standard error.

### *Reagents and instruments*

The solutions of 2,3,5-triphenyltetrazolium chloride; phosphate buffer solution with *p*H 7.4, isocitric acid, α-ketoglutaric acid, succinic acid and malic acid; glucose were prepared using reagents of the highest analytical

* email: ciornea@uaic.ro

purity. The spectrophotometric analyses were performed using a UV-Vis Shimadzu 1700 spectrophotometer, in 1 cm quartz cuvettes, toward a reagents control.

*Principle of the methods*

The method used to evaluate the Krebs cycle dehydrogenases activity from *Capsicum* foliar tissue and is based on the capacity of these enzymes to transfer the hydrogen from different substrates, such as carboxylic acids, alcohols, carbohydrates etc., to 2,3,5-triphenyltetrazolium chloride which is reduced to triphenyl-formazan with a red color, the color intensity of this compound being proportional with dehydrogenases activity [11]. The microbiological activity of the soil, rated through the actual and potential dehydrogenase activity, was highlighted through Casida method [12].

The assimilatory pigments content were assessed using a portable SPAD (Minolta Co.) device. Measurements were performed on 5 leaves per each variant. Chlorophyll fluorescence was measured with a FMS2 portable fluorometer (Hansatech Ltd.). The actual quantum yield of photosystem II (ΦPSII) is a light-dependent fluorescence parameter [13] and was determined on 5 leaves for each variant.

*Statistics*

Statistical processing of the experimental data obtained was performed using Graphpad Prism 7, the data being expressed as average values for each batch ± standard error, the ANOVA and Tukey tests being used to assess variance of values and statistical significance.

**Results and discussions**

It is well known that the economic development led to the division of society into various sequences: initially, the rural period, the industrial one and then, today's modern period, which has been submitted to transformation by the dominant economic sector - agricultural or industrial [14]. The modernization of entire society entailed the development of industrial technologies and modernization of agriculture practices, which involved, among other things, massive accumulation of heavy metals and various industrial waste in the water and soil, but also increased the use of chemical substances such as fertilizers, pesticides, insecticides, acaricides etc., that induce extremely negative effects on long term, leading to massive pollution of the environment and loss of biodiversity with negative impact on human and animal health [15,16].

The scientific literature reveals the various effects of pollutants that cause a highly oxidative stress in tissues and vegetal organs, translated in the emergence of some major imbalances at cellular level [17,18], therefore assessing the impact produced by combinations of metal ions in different concentration in plants havinng particular importance [19,20].

The use of DNP, Atonik and heavy metal salts treatments on Macska sarga cultivar of *Capsicum annuum* and Aji Amarillo cultivar of *Capsicum baccatum* var. *pendulum*, led to different results concerning the activity of foliar dehydrogenases, but also of the content of assimilating pigments and chlorophyll fluorescence. Thereby, the isocitrate-dehydrogenase activity was significantly higher than in control plants for Macska sarga individuals treated with $NiCl_2$ and $(CH_3COO)_2Pb$ (22.5236 ± 0.423 and 22.7712 ± 0.51 µg formazan/g fresh tissue, compared to 5.459 ± 0.35 µg formazan/g fresh tissue), respectively Atonik and $NiCl_2$ for Aji Amarillo variety (16.359 ± 0.4 and 15.335 ± 0.31 µg formazan/g fresh tissue compared to 9.141 ± 0.3 µg formazan/g fresh tissue) (fig. 2).

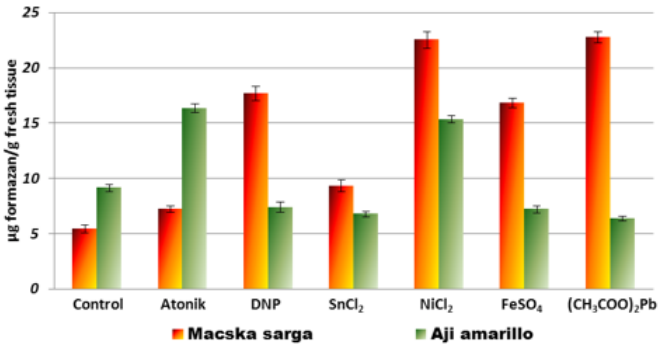


Fig. 2. Isocitrate-dehydrogenase activity in *Capsicum annuum* cv. Macska sarga and *Capsicum baccatum pendulum* cv. Aji Amarillo

Atonik represents a synthetic product that increases plant resistance to stress factors [21], and enters the plant shortly after the application, where it causes activation of cytoplasmic flow and increases the circulation of raw sap and of the substances assimilated in soil, determining a stimulatory effect on aerial vegetative growth and root growth and increasing also the flower fertility, and stimulating the elongation of the pollen tube and the germination of pollen grains [22]. On the other hand, the scientific literature highlights that nickel, alongside with other metals like Cd, Cr, Zn and Pb, is involved into a great variety of cell processes [23], its excess concentration determining toxicity in many organisms, including superior plants [24]. The occurrence of wilting symptoms and leaf chlorosis [25], growth inhibition and also the inhibition of dry substance production [26] as well as impaired absorption of nutrients such as potassium, calcium and magnesium in the various parts of the plant [27], but also a decrease in the flower and fruits number and thus the yield of different plant species [28] are assigned to the presence of Pb in the substrate.

*Capsicum annuum* cv. Macska sarga presented keto-glutarate dehydrogenase activity (fig. 3) with values of 4.538 ± 0.31 mg formazan / g fresh tissue for the experimental variant treated with FeSO4, 6.349 and 6.794 mg formazan / g fresh tissue for the samples treated with DNP and $(CH_3COO)_2Pb$, respectively 10.886 ± 0.6 and 11.481 mg formazan / g fresh tissue in the version treated with Atonik and the control sample, whereas *Capsicum baccatum* var. *pendulum* cv. Aji Amarillo, behaves slightly different; in this case, the sample treated with DNP presents the lowest activity of keto-glutarate dehydrogenase (7.338±0.35 µg formazan/g fresh tissue), followed by the samples treated with $(CH_3COO)_2Pb$ and $SnCl_2$ (7.545 ± 0.302 and 9.572 ± 0.4 µg formazan/g fresh tissue, respectively), while the control and Atonik samples present again the highest activity, the registered values being however almost 3 times higher compared to Macska sarga

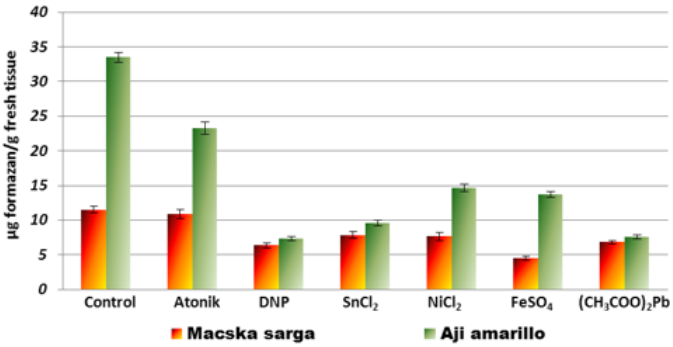


Fig. 3. Keto-glutarate dehydrogenase activity on *Capsicum annuum* species, Macska sarga variety and *Capsicum baccatum pendulum* species, Aji Amarillo variety

variety (33.468 ± 0.74 and 23.265 ± 0.84 µg formazan/g fresh tissue).

There is no clear evidence that tin would be essential for plants, although it seems that small amounts of tin chloride alongside with other elements, induce a favorable response. The data from scientific literature [29,30] reveal plant's ability to assimilate and accumulate the tin found in the environment due to rocks degradation and volcanic eruptions, but also due to anthropic activities such as industrial processes or agricultural and mining technics [31]. Besides that, it is considered that Sn excess may be toxic for superior plants. At the same time, scientific evidences indicate that pepper plants are very sensitive to the presence of lead in growth media [32,33]. The toxic effects of this metal described at sub-cellular level are: respiratory and photosynthetic inhibition due to disruption of the electron transfer reaction, effects on mitosis and water absorption, deterioration of mineral nutrition etc., which cause change of hormonal statute and affect the membrane's structure and its permeability for water, determining the inhibition of plant's growth [34,7].

The dehydrogenation rate of succinic acid to form fumaric acid under the action of succinate-dehydrogenase (fig. 4) differs depending on the type of treatment applied on peppers. Thereby, the enzyme's activity is greater for the control sample and for the sample treated with the growth stimulator containing sodium nitrophenolate (about 12 µg formazan/g fresh tissue for Macska sarga and 11.326 ± 0.64 µg formazan/g fresh tissue for Aji Amarillo). The lowest activity limits were detected for samples treated with $(CH_3COO)_2Pb$, $SnCl_2$ and $NiCl_2$ from Macska sarga (2.846, 3.002 and 3.345 µg formazan/g fresh tissue) and DNP and $(CH_3COO)_2Pb$ respectively from Aji Amarillo (5.359 and 5.454 µg formazan/g fresh tissue).

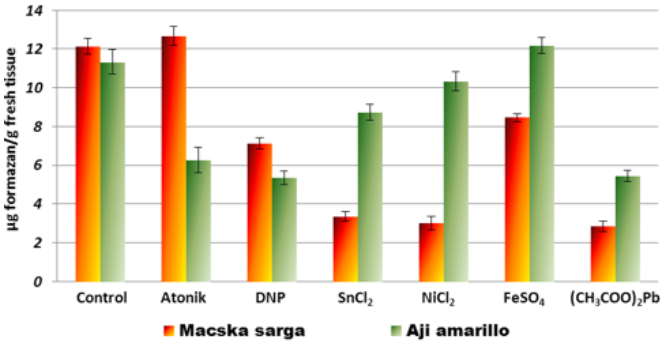


Fig. 4. Succinate-dehydrogenase activity in *Capsicum annuum* cv. Macska sarga and *Capsicum baccatum pendulum* cv. Aji Amarillo

Malate's mobility speed in the presence of malate-dehydrogenase, via oxidation reaction coupled with the reduction of $NAD^+$ and formation of oxalo-acetate, ranges between 2.936 ± 0.21 µg formazan/g fresh tissue (sample treated with $FeSO_4$) and 11.326 ± 0.48 µg formazan / g fresh tissue (Atonik sample) for Macska sarga variety, while for Aji Amarillo variety, the enzyme activity ranges between 4.454 ± 0.32 µg formazan / g fresh tissue (for the sample treated with $(CH_3COO)_2Pb$) and 10.389 ± 0.35 µg formazan/g fresh tissue (in case of DNP solution) (fig. 5).

Considering the results, it should be noted that there is a close correlation between the high amount of malate and the activity of phosphoenolpyruvate-carboxylase involved in basic cellular metabolism during stomatal opening and also in providing malic acid as a respiratory substrate [35] in vegetal tissues exposed to various stress conditions.

The values of assimilatory pigments contents in leaves did not show significant differences in control plants compared to the treated ones (fig. 6). Chlorophyll

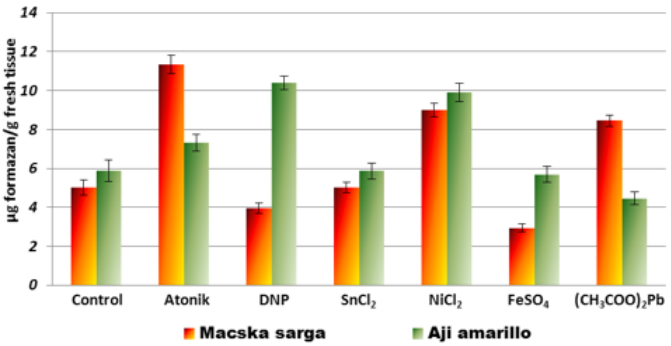


Fig. 5. Malate-dehydrogenase activity in *Capsicum annuum* cv. Macska sarga and *Capsicum baccatum pendulum* cv. Aji Amarillo

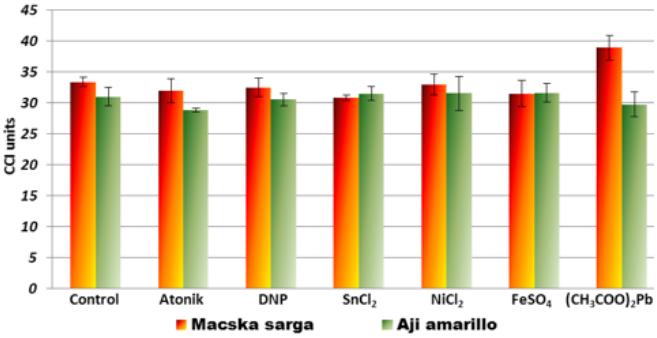


Fig. 6. The content of leaf assimilatory pigments in *Capsicum* cultivars

fluorescence under light regime was also not significantly influenced by the treatments (fig. 7), however different responses for the cultivars were observed. The Macska sarga cultivars plants registered increased ΦPSII values under all treatments compared to controls, while in Aji amarillo plants ΦPSII values were lower than in control plants.

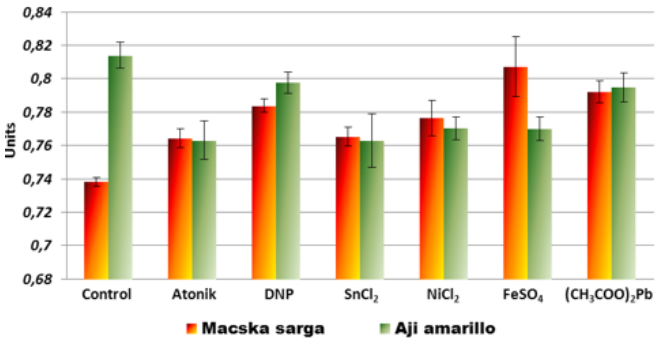


Fig. 7. Chlorophyll fluorescence (ΦPSII) values in *Capsicum* cultivars leaves

Chlorophyll content and fluorescence values indicate that the photosynthetic apparatus was not significantly affected by metal presence. While it is known that chlorophyll fluorescence is a sensitive parameter to various types of stress [36], the effective quantum yield of the photosystem II describes the efficiency of light use for transport and is related to the $CO_2$ assimilation in photosynthetic tissues [37]. However, the effects of certain metals on the photosynthetic apparatus are concentration dependent, and may reduce photosynthetic activity at high concentrations, as does Ni [38] or Pb [39]. The chlorophyll contents and chlorophyll fluorescence may register variations due to metal presence, but mechanisms other than photosynthetic ones may become limiting factors under low metal concentrations [40,41]. The differences in chlorophyll related parameters recorded by the two cultivars may be assigned to genetic differences, as noted previously [42].

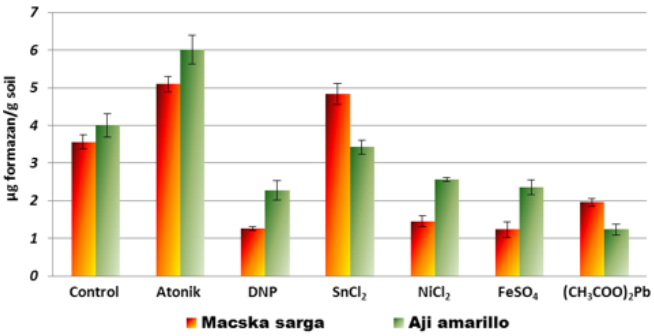


Fig. 8. Current soil dehydrogenases activity

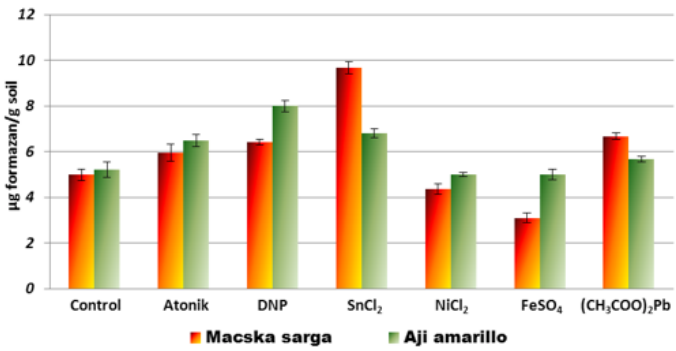


Fig. 9. Potential soil dehydrogenases activity in *Capsicum* cultivars plants

A final objective of this study was to determine the activity of dehydrogenases in soil samples, knowing that the soil's microbial activity can be strongly influenced by both natural disturbances and anthropogenic activities, and therefore the value of the enzyme activity indicates a fast response to these changes [43].

As noted in figure 8, for both pepper varieties used in this study, current dehydrogenase activity is significantly higher in soil samples from the experimental variant treated with Atonik (5.098 ± 0.21 mg formazan / g soil for Macska Sarga variety and 0.39 ± 6.012 mg formazan / g soil for Aji amarillo variety), followed by control sample (3.562 ± 0.18 mg formazan / g soil and 4.002 ± 0.32 mg formazan / g soil, respectively) and $SnCl_2$ sample 4.836 ± formazan 0.27 mg / g soil and 3.422 ± 0.19 mg formazan / g soil, respectively).

Regarding the potential activity (fig. 9), it can be remarked on the one hand the presence of much higher values compared to current dehydrogenases activity, and on the other hand that, except $NiCl_2$ and $FeSO_4$ variants, enzymatic activity from all other samples is superior to the control batch (9.658 ± 0.27 µg formazan / g soil for Macska sarga samples treated with $SnCl_2$, 7.987 ± 0.25 µg formazan /g soil for Aji amarillo treated with DNP, 6.669 ± 0.15 µg formazan / g soil and 5.659 ± 0.13 µg formazan / g soil for experimental variants treated with $(CH3COO)_2Pb$).

From the ratio between potential/actual dehydrogenase it can be noted that the soil dehydrogenation potential is about 1.1 higher in the batches treated with Atonik, 1.4 and 1.99 respectively, higher in control samples and those treated with $SnCl_2$, while, at the opposite side, the variants treated with $(CH_3COO)_2Pb$ and DNP presented remarkable potential dehydrogenase activities, in this case the registered difference being 4.57 times higher (Aji Amarillo -Pb) and 5.041 times higher, respectively (Macska sarga – DNP).

Our data concords with the data from literature which indicate that a number of factors such as maintenance and management practices, soil fertilization, the presence of varied heavy metals etc. have a major effect on dehydrogenase activity in soil, which is increasing with the pollution degree [44-46].

## Conclusions

Applying different treatment types on the genus *Capsicum*, Macska Sarga and Aji Amarillo cv., exerted a marked influence on the activity of Krebs cycle dehydrogenases in tissue foliar as well as those from the soil, while the content of chlorophyll and values of fluorescence were not visible affected, requiring in-depth studies on the influence of these organic and inorganic substances on various physiological and biochemical processes.

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