CONTROLLED RELEASE OF PESTICIDES INTERCALATED IN LHD AND CEREAL PLANT GROWTH

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The most well-known anionic clays are the hydrotalcite-like layered double hydroxides (LDHs). Different minerals have been utilized to generate a bewildering array of materials, all of which function as anionic clays. The anionic clays exhibit anion sorption, anion diffusion and exchange properties together with surface basicity making them materials of importance for many modern applications. These anionic clays are useful in agriculture due their physical and chemical properties, which decrease the pollution effects, in order to obtain organic products. The present study has been carried out to develop the potential of LDH as plant nutrients, pesticides and growth regulators of plants. It is known that serious environmental problems arise from the use of pesticides. Application of LDH in pesticide formulations is currently attracting interest. To increase pesticide efficiency and to reduce their leaching into the air and water, the intercalation into layers of LDH is a good solution, because the LDH effectively protect unstable pesticides against volatilization and photo-degradation, which lead to increasing frequency and dose of herbicide treatment. In this work, a study concerning the effects of some nanocomposites (anionic clay containing the pesticid chlorothalonil) on plant growth is presented. Seeds of corn (Zea mays) and wheat (Triticum sativum) were put into Petri dishes on double filter paper together with suspensions from these nanocomposites. Then the germinated seeds from control variant and treated ones are planted in boxex and they developed in Biophysics Department Laboratory. The dynamic of germination and the growth has been monitorized during the first phenophase of growth. The content of photosynthetic pigmens and the aminoacid content from plant leaves has been determined spectrophotometrically. Our results showed that the nanocomposites could modify the plant growth by controlled release of the pesticide.

***Key words**: LDHs, chlorothalonil, pigment content, alanine.*

Mesoporous synthetic clays (MSCs) are obtained when polymer-containing silicate gels are hydrothermally crystalized to form layered magnesium silicate hectorite clays containing polymers that are incorporated in situ [1]. Layered double hydroxides (LDHs) or the so-called anionic clays are the analogue of the extensively studied family of cationic smectite clay minerals, a group of minerals which tend to fill out when they are inserted into water (bentonite includes minerals from smectite group). LDH structure is described with formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_{2}][A^{n-}_{x/n}.zH_2O]$, where M is a metal cation and A is the anion.

The anionic clays exhibit anion sorption, anion diffusion and exchange properties together with surface basicity making them materials of importance for many modern applications [2], [5], [9].

Recent reports on the advantages of mesoporous materials as drug delivery vehicles have imposed research in novel applications and several materials with this purpose have been reported [8]. The potential of mesoporous materials to improve the permeability of large hydrophilic drug substances has also been explored. Due to their high anion exchange capacities, LDHs have been investigated as potential adsorbents for removal of anionic contaminants from aqueous systems as arsenite from water [12]. M. Lakraimi and coworkers [6] studied the ion exchange from the molecule of pesticide 2.4-dichlorophenoxyacetate (2.4D), the paraquat, by the anionic clay [Zn-Al-Cl] using X rays and IR spectroscopy.

On the other hand, very few studies have been conducted to assess potential toxicity of nanomaterials to ecological terrestrial test species (plants, wildlife, soil invertebrates, or soil microorganisms). Limited studies reported both positive and negative effects of nanoparticles on higher plants. It was pointed out that some nanoparticles enhance the abilities of absorbing and utilizing water and fertilizer and apparently hasten its germination and growth. By contrary, the toxicity of nanoparticles may be attributed to two different actions a) a chemical toxicity based on the chemical composition, e.g., release of (toxic) ions; and (b) stress or stimuli caused by the surface, size and/or shape of the particles [7]. However, there are still many unresolved issues and challenges concerning the biological effects of nanoparticles.

The present study has been carried out to develop the potential of LDH as plant nutrients, pesticides and growth regulators of plants. It is known that serious environmental problems arise from the use of pesticides. Application of LDH in pesticide formulations is currently attracting interest. To increase pesticide efficiency and to reduce their leaching into the air and water, the intercalation into layers of LDH is a good solution, because the LDH effectively protect unstable pesticides against volatilization and photo-degradation, which lead to increasing frequency and dose of herbicide treatment. The plant growth regulators also may be intercalated in LDH through co-precipitation or reconstruction methods to explore the protected storage and controlled release in natural environments. Were prepared various layered double hydroxids: MgAlLDH, CuAlLDH, BaAlLDH, free, intercalated with Fe_3O_4, with Atonik, chlorothalonil (CTL), salicylic acid and nano-composites obtained by immobilization of magnetite and bio-compounds in the structure of the LDH. Nanocomposites LDH-CTL and LDH-CTL-Fe_3O_4 were prepared by reconstruction method, because chlorothalonil is stable only to pH 7. In this paper, the comparative effects of these nanocomposites on germination rate, root elongation and growth of corn *(Zea mays)* and wheat *(Triticum sativum)* were analyzed. Germination rate and root elongation, as a rapid phytotoxicity test method, possess several advantages, such as sensitivity, simplicity, low cost and suitability for unstable chemicals or samples. These advantages made them suitable to study mechanisms of phytotoxicity [10], [11].

Chlorothalonil, is the active substance of Bravo 500C fungicide prodced by Syngenta Limited Anglia and used for control fungi *Phytofara infestans,Alternaria solani*, *Septoria lycopersici*, etc., that threaten vegetables, trees, and other agricultural crops.

Chlorothalonil known as 2,4,5,6 tetrachloro-1,3benzenedicarbonitrile is a chemical compound having cancerigen potential.

MATERIAL AND METHOD

The experiments were conducted in the Biophysics Department Laboratory and in the greenhouse of the University of Agricultural Sciences and Veterinary Medicine, Iasi, Romania. As a biological material we used two species of cereals, studied by many authors, corn (*Zea mays*), and wheat (*Triticum sativum*), the most widely used cereals in our country. Seeds of corn and wheat were put into Petri dishes on double filter paper together with 5 mL treatment solution (a suspension that contains 0.5g of clay and 50mL bidistilled water). Here the seeds were kept in dark at the optimal temperature (23^0C) for 3 days. Every day we poured bidistilled water for control and treatment solution for the other variants to determine seed germination.

The dynamic of germination and the growth has been monitorized during the first phenophase of growth. After that the germinated seed were planted in soil in boxex. Photosynthetic pigments were extracted in acetone [3], measured spectrophotometrically using a spectrophotometer SPECORD 200 produced by Analytik Iena and calculated according to Lichtenthaler formula. The content of plant aminoacids was determined using a simple method based on the spectrophotometric analysis developed by Drochioiu and coworkers [4].

To study the effect of anionic clays on plant growth, four clays have been prepared and we sorted the following variants:

1. control;
2. chlorothalonil (20mg/1000ml distilled water);
3. MgAl LDH;
4. MgAl LDH-CTL;
5. MgAl LDH-Fe_3O_4;
6. MgAl LDH-CTL-Fe_3O_4.

RESULTS AND DISCUSSION

In *figure 1* the wheat seed germination for control and treatment with chlorothalonil is presented. From *figure 1* we can see that the germinated wheat seeds have more developed roots than the control but these roots are very thin'. On the other hand the stems have been developed faster for control plantlets and green coloratura appeared for these plantlets.

Figure 1 **Wheat seed germination after 3 days**

Figure 2 **Corn seed germination after 3 days**

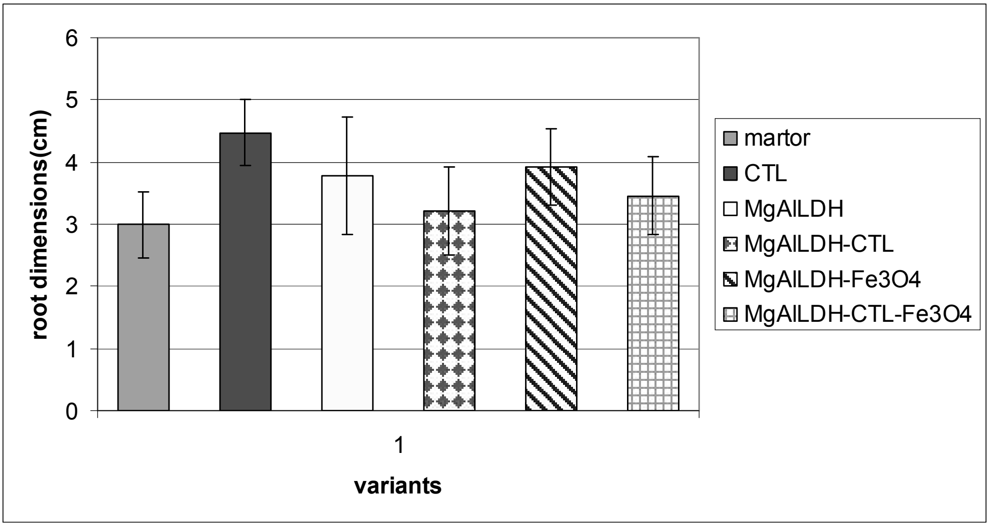

Figure 3 **Corn root dimensions after 3 days of treatment. Error bars are 95% confidence intervals and n=20**

Plants were grown for 8 days. Then plants were harvested and photosynthetic pigments were measured as in [3]. Content of photosynthetic pigments, chlorophyll a (Cha), clorophyls b (Chb) and carotenoids (Car) from corn leaves are given in *figure 4.*

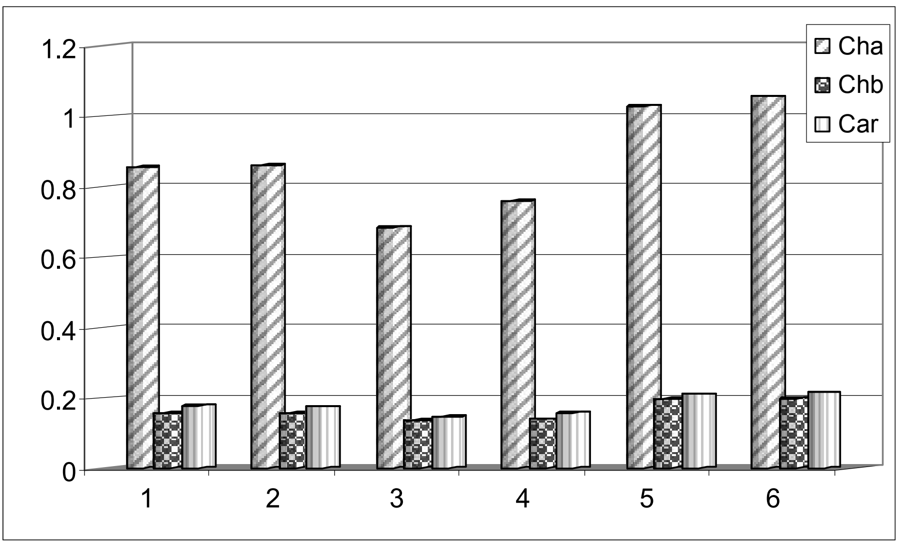

Figure 4 **Content of photosynthetic pigments from corn leaves (mg/g fresh tissue)**

This figure shows that the treatment with clay containing magnetite, free or with chlotothalonil, by comparison with the other clay, increases pigment content from corn leaves.

The plant biostructure can be studied measuring the content of released aminoacids. In figure 5 and figure 6 the content of alanine from wheat and corn leaves respectively is given.

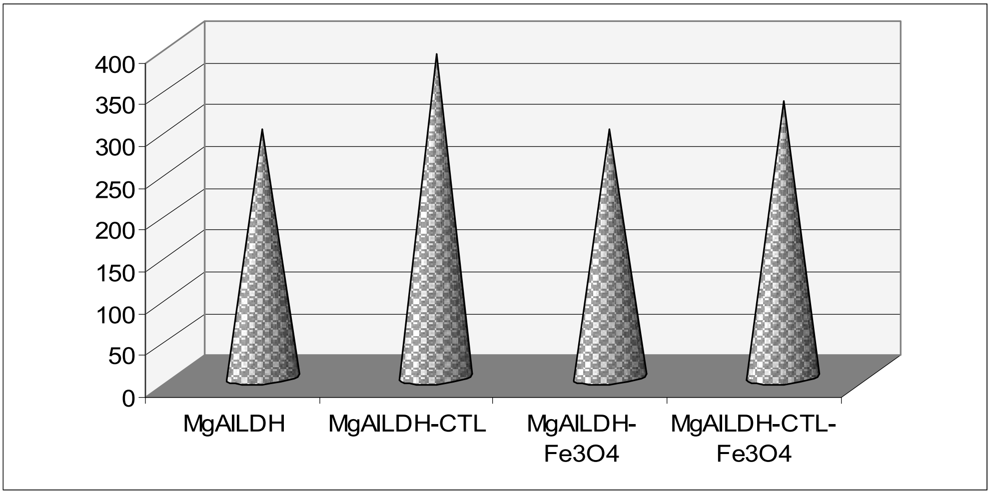

Figure 5 **Content of aminoacids from *Triticum aestivum* leaves (mg/100g fresh tissue)**

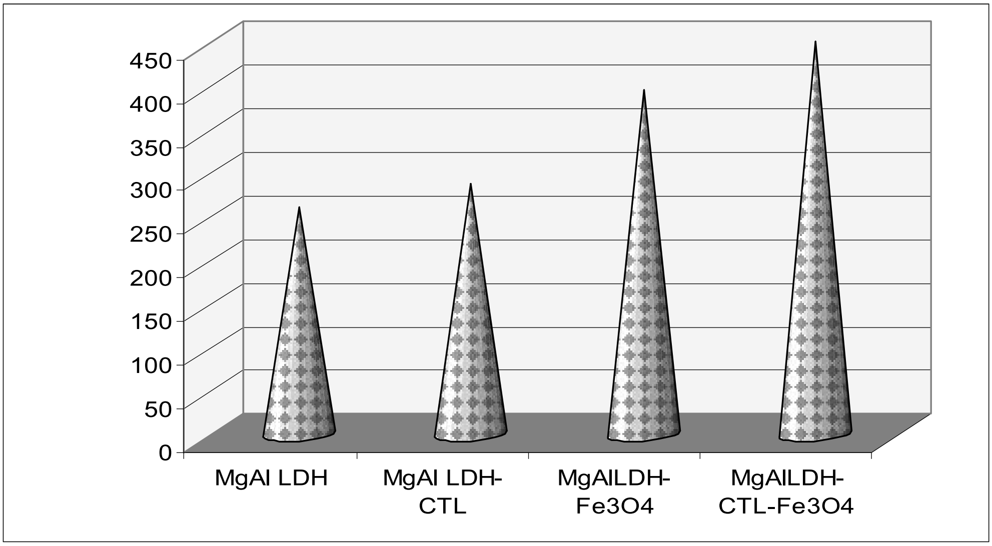

Figure 6 **Content of aminoacids from *Zea mays* leaves (mg/100g fresh tissue)**

From these figures we can see that chlorothalonil intercalated in LDHs increases the content of aminoacids, especially in the case of LDH containing magnetite.

CONCLUSIONS

Our results prove that the highest differences between variants appear for root plants and this is because the roots developed in the anionic clay suspensions. The strongest increase of the plant roots has been observed for the seeds treated with chlorothalonil but the roots have been very thin. By contrary the stem developed faster for control than the treated one. After seed planting, if the plants developed in the same conditions, the effects have been decreased but the clay containing magnetite with or without chlorothalonil yet increses the pigment content and aminoacid content. This means we need to have this procedure repeated in the future. Therefore the anionic clays are substances which might use in agriculture in order to reduce the pesticide leaching into the air and water. Due to a pesticide property of chlorothalonil, a slow release from its nanocomposite material can be exploited for control release formulation of the pesticide; this means the intercalation of pesticides into layers of LDH is a feasible solution.

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