

# The Effect of Soil pH and Cadmium on Nitrogen, Phosphorus and Chlorophyll Contents in Corn (*Zea mays* L.)

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## Abstract

A pot experiment was carried out in the plastic greenhouse at the Institute of Sustainable Agrotechnology (INSAT), University Malaysia Perlis in Sungai Chuchuh, Perlis, Malaysia during the season of cultivation of 2014 to investigate the impact of soil pH and cadmium (Cd) on nitrogen (N), phosphorus (P) and chlorophyll contents of corn plant (*Zea mays* L.). Twenty-five were arranged in factorial experiments according to the complete randomized design (CRD), with three replicates. Five levels of soil pH were: pH4, pH5.2 (i.e., the original value), pH6, pH7 and pH8 and five levels of Cd: Cd 1, Cd 2, Cd 3, Cd 4 and Cd 0 where the amounts (2, 4, 6, 8 mg. kg<sup>-1</sup> soil and control treatment without add Cd) are applied as CdCl<sub>2</sub>. Thus, the total numbers of pots were 75 pots. The results showed the soil pH7 led to significant effect in increasing the N and chlorophyll contents, while the decrease of soil pH to 5.2 led to significant effect to increase P content in the corn, as well as increase concentration of Cd led to significant decreasing in N, P and chlorophyll contents. As for the interactions between soil pH and Cd, it led interaction between pH 4 and Cd 4 to decrease of the N and chlorophyll contents in the corn, while the interaction between pH 8 and Cd 4 led to decrease in P contents in the corn.

**Keywords:** pH of soil, Cadmium, Nitrogen, Phosphorus, Chlorophyll, Corn, Perlis

## Introduction

Soil pH is one of the important factors affecting soil fertility and plant nutrition because of its large contribution in the soil's ability to process the correct ratio of nutrients needed by the developing plant. Soil pH is also one of the important indicators to the need of soil of the nutrients during fertilization process to reclaim and improve soil fertility to meet the plant nutrient needs. Soil pH can impact the growth of a plant based on its influence on the availability of essential plant nutrients and also on the concentration of toxic elements in the plants.<sup>28</sup>

Pass work shows that the increase of soil pH value has led to slight rise in the soil alkaline hydrolysable N - available and P.<sup>29</sup> Puga et al.<sup>21</sup> report that the addition of biochar to the soil has led to the increase in pH (about 0.3 units). This has caused the N, P, K, and sulfur (S) concentrations increase in shoots of jack bean and *Mucuna atterima* with an increasing rate of biochar. Liu et al.<sup>16</sup> report that the chlorophyll content was higher under soil pH 5.5 than under soil pH 7.5 and 8.0; also that the soil pH 5.5 was most appropriate for P. This result agrees with Cregg,<sup>7</sup> who pointed out that the increasing media pH significantly reduced needle chlorophyll content in all of the species tested.

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Rahat et al.<sup>23</sup> said that the mineral deficiencies or imbalances and depression of plant growth can result from excessive Cd toxicity that affects the rate of uptake and distribution of certain nutrients in plants. Soil contamination with Cd resulted in a decrease of N content in the above-ground parts of *Zea mays*.<sup>6</sup> Cd accumulation alters mineral nutrient uptake and inhibits stomatal opening by interacting with the water balance of the plant.<sup>11</sup>

Plant pigments such as chlorophyll-a, chlorophyll-b and total carotenoids get decreased with the increasing of Cd soil addition.<sup>4</sup> Cherif et al.<sup>5</sup> agree with this result as they found that photosynthetic pigments showed a drastic reduction (50%, 28% and 45% of Chlorophyll-a, Chlorophyll-b and carotenoids, respectively) in 10 micromol/L Cd-treated plants in comparison to the control.

Corn (or maize) is one of the oldest human-domesticated plants. Known as the third largest planted crop in the world after wheat and rice, it is mostly used as a primary feed crop – for instance, it accounts for 95% of the total food grain production and its use in the United States. Corn is also important as a food crop in many parts of the world, and in food processing for making starch, sweeteners, oil and beverage, etc. Besides food and feed, nowadays corn has been playing an important role in industrial ethanol production.<sup>22,26</sup>

## Materials and Methods

### Experimental Design and Treatments

Agricultural experiment is performed inside the plastic greenhouse and arranged by factorial experiments according to the completely randomized design (CRD), with three replicates. This experiment consisted of five levels of pH value in the soil (pH 4, pH 5.2, pH 6, pH 7 and pH 8) and five levels of Cd (Cd 1, Cd 2, Cd 3, Cd 4 and Cd 0) where the amounts (2, 4, 6, 8 mg. kg<sup>-1</sup> soil and control treatment without add Cd) were applied as CdCl<sub>2</sub>. The five of corn plants were planted for each treatment in pots 10 kg, which were packed with sandy loam soil, and irrigated daily by a drip-irrigation system. Where reached the number of treatments for each factor of the study factors (5×5×3).

### Management of the Experiment

Soil preparation was done before two weeks of cultivation. Soil pH was adjusted to 4.0, 5.2 (i.e., the original value), 6.0,

7.0, and 8.0 by adding a 2 ml solution of 0.15 M HCl, distilled H<sub>2</sub>O and 0.06 M, 0.15 M, and 0.6 M NaOH, respectively to 20 g of soil aerobically dried.<sup>30</sup> A complete amount of 10 kg soil was used for each treatment. After that, all the amount of Cd was added to the soil and was mixed together for full homogeneous and then put in the pots (10 kg). Then, the pots were irrigated with water and incubated for two weeks before transferring corn seedlings for guaranteed uniform distribution of Cd. Ahmed et al.<sup>3</sup> recommends right quantities of NPK fertilizer. A fertilizer rate of 60 N kg.ha<sup>-1</sup>, 60 P kg.ha<sup>-1</sup> and 40 K kg.ha<sup>-1</sup> for the maize was followed, MARDI (Malaysia Agriculture Research Development Institute). The fertilizers used were urea (46% N), CIRP (30% P<sub>2</sub>O<sub>5</sub>) and MOP (60% K<sub>2</sub>O). Each seedling was transferred from transplant tray to each pot, which contained 10 kg of soil. All the agronomic practices like weeding; irrigation and plant protection measures were performed as and when necessary. These plants were allowed to grow till they hit the maturity level. All the plants were planted on 10th of June 2014 and were harvested after 110 days.

### Nitrogen Content in Dry Matter of Corn Plant (Aerial Part) (%)

Total N content in the plant was determined by the following method. A sample of 0.2 g of finally ground plant material was digested with 4.4 mL of digestion mixture (selenium powder, lithium sulfate) at 380°C for 2 hours. After digestion, volume of digest was made to 100 mL. A 0.1 mL of digest was treated with 5 mL of N1 reagent (sodium salicylate, sodium citrate and sodium tartrate) and reagent N2 (sodium hydroxide and sodium hypochlorite), kept for 1 hour for color development. The intensity of yellow color was determined at 655 nm wavelength using spectrophotometer.<sup>9</sup>

### Concentration of Phosphorus in Corn Plant (Aerial Part) (mg. kg<sup>-1</sup>)

For P determination, 1 mL of the extract of wet digestion method was taken into a 10-mL tube. 2 mL of 1.0 N HNO<sub>3</sub> was added and diluted to 8 mL with distilled water. To tube containing extract, 1 mL of molybdate vanadate solution was added to 10 mL with distilled water. Tube was shaken and allowed to stand for 20 min. The absorbance was measured on spectrophotometer at 430 nm wavelength and was compared with the absorbance of the standard phosphorus curve.<sup>9</sup>

**Table 1. Initial Physical and Chemical Properties for Soil of Study**

Bulk Density (g/cm <sup>3</sup> )	pH	CEC (cmol. kg <sup>-1</sup> )	EC (dS. m <sup>-1</sup> )	Cd (mg.kg <sup>-1</sup> )	N (%)	P (mg.kg <sup>-1</sup> )	K (mg.kg <sup>-1</sup> )	Ca (mg.kg <sup>-1</sup> )	O. M (%)
2.3	5.20	2.03	0.24	0.92	1.24	37	95.7	6.4	0.21

## Chlorophyll Content

It was measured with the help of a portable SPAD-502 Plus chlorophyll meter. All the readings were done at 10:00 to 11:00 a.m.<sup>2</sup>

## Statistical Analysis

Data were subjected to statistical analysis using the statistical software GenStat.<sup>20</sup> Variety ANOVA was used to analyze the experimental results of dependent variables (treatments). The least significant difference (LSD) was calculated at  $P \leq 0.05$ .

## Results and Discussion

### N Content in the Plants' Dry Matter (Aerial Part) (%)

Figure 1 explains the superiority of pH 7 substantially on all other levels in increasing the content of N in the plants' dry matter, where it reported the highest content reaching 0.43%, whereas pH 4 resulted in the lowest of content reached 0.19%, which is most likely caused by the presence of N in the neutral soil, where its absorption decreases due to a decrease in the pH of the soil.<sup>24</sup>

In terms of the influence of Cd levels, the results indicated that the increase of Cd concentration in the soil led to a decrease of the content of N in the plants' dry matter, resulting in the lowest content 0.17% at Cd 4, as opposed to Cd 0 that resulted in the highest content 0.46%, as shown in Fig. 2. These results agree with Nwugo and Huerta,<sup>18</sup> who pointed out that the increase of Cd concentration in soil led to a decrease in the nutrients, due to the inhibition in photosynthesis that inhibit the activity of the main enzyme of the Calvin Cycle, and inhibiting at the plant from absorbing nutrients. Cd will interact with the absorbed nutrients by changing the permeability of the plasma membrane, which leaks the nutrients.<sup>23</sup> This affects the process of transferring nutrients via the membrane.

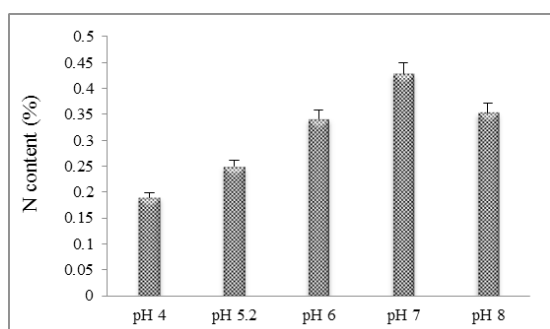


Figure 1. Effect of Soil pH Levels on N Content (%)

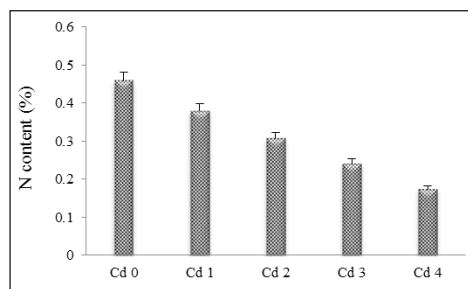


Figure 2. Effect of Cadmium Levels on N Content s (%)

The interaction between the soil pH and Cd led to big differences in the N content in the plants' dry matter, where superior interaction between pH 7 and Cd 0 is dramatically higher compared to other interactions, resulting in the highest nitrogen content, which was 0.57%, while the interaction between pH 4 and Cd 4 resulted in the lowest content, reaching 0.06% from the plants' dry matter (Fig. 3). This is most likely caused by the influence of high soil acid effect on the increase of Cd's availability, which led to absorption by the plant without absorbing N.<sup>1</sup>

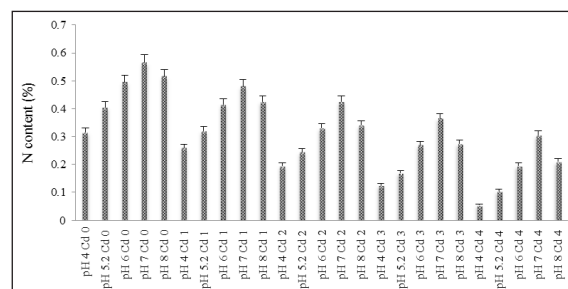


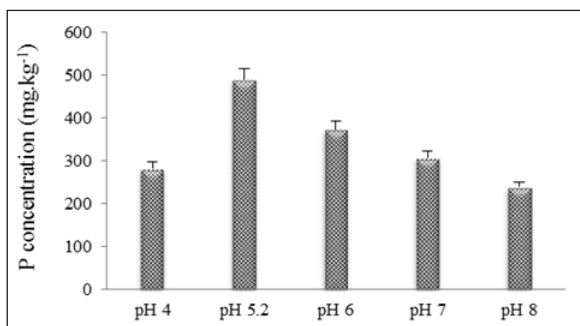
Figure 3. Effect of Interaction between Soil pH and Cadmium Levels N Content s (%)

### P Concentration in the Plant (mg.kg<sup>-1</sup>)

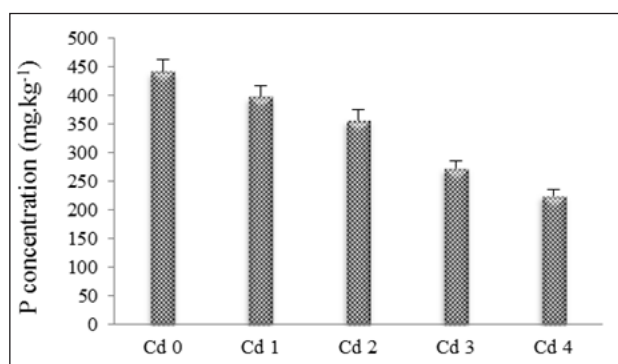
The results in Fig. 4 indicate that there is a big difference between the levels of soil pH in absorption and accumulation of phosphorus in plants, where a pH 5.2 showed high superiority over the rest of the soil pH levels in the study, where it reached 490.66 mg.kg<sup>-1</sup>, while the lowest concentration was 239.38 mg.kg<sup>-1</sup> at pH 8. This is most likely caused by the presence of this element at this level of the soil's acidity,<sup>24</sup> while decreasing the soil's pH under this level led to a decrease in the absorption of the same element by the plant.

Figure 5 indicates an inverse relationship between the absorption of P in the plant and the level of Cd in the soil. Cd 0 resulted in the highest concentration of P in the plant, reaching 441.88 mg.kg<sup>-1</sup>, while it decreased when the concentration of Cd in the soil increased to 225.26 mg.kg<sup>-1</sup> at a level of Cd 4. This decrease might be caused

by the availability of Cd for absorption and transfer to the plant, and consequently lack of other mineral nutrients in the plant.<sup>10,23</sup>

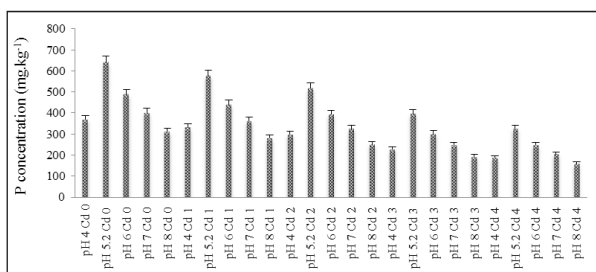


**Figure 4. Effect of Soil pH Levels on P Concentration in the Plant (mg.kg<sup>-1</sup>)**



**Figure 5. Effect of Cadmium Levels on P Concentration in the Plant (mg.kg<sup>-1</sup>)**

On the other hand, the interaction between the soil's pH levels and Cd levels led to big differences in the concentration of P in the plant, where the superiority of the interaction between pH 5.2 and Cd 0 is evident over other interactions in increased P concentration, where the highest concentration reached 639.63 mg.kg<sup>-1</sup>, while the interaction between pH 8 and Cd 4 resulted in the lowest concentration for the P reached 188.13 mg.kg<sup>-1</sup>, as shown in Fig. 6. This is caused by P not being absorbed by the plant at higher levels of soil pH and availability of Cd in high concentrations, which led to its absorption and accumulation in the plant more than P.



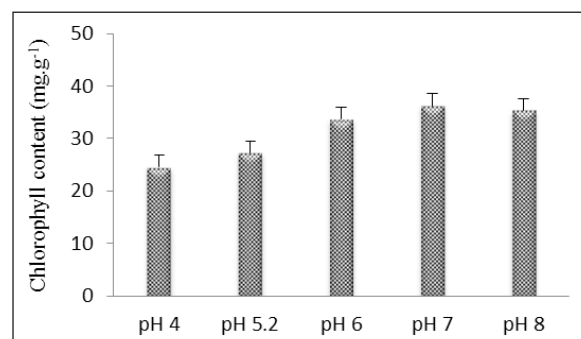
**Figure 6. Effect of Interaction between Soil pH and Cadmium on P Concentration in the Plant (mg.kg<sup>-1</sup>)**

## Chlorophyll Content in Leaves (mg.g<sup>-1</sup>)

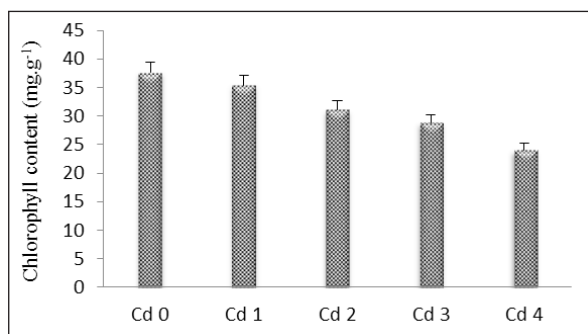
The level of pH 7 is one of the well-studied levels of soil that substantially influences the increase of the chlorophyll content in leaves reaching 36.74 mg.g<sup>-1</sup>. It is remarkably superior to the rest of the levels. Figure 7 shows that the chlorophyll content has decreased with decreasing pH until it reached the lowest rate of 24.73 mg.g<sup>-1</sup> at pH 4. This might be due to the non-readiness of N in the soil at this level and the plants' inability to absorb it, leading to the accumulation of chlorophyll in the plant with magnesium that will be ready at a soil pH 6–7.<sup>15,17</sup>

The Cd led to decreased chlorophyll content in the leaves of the corn substantially at Cd 4 compared to all other levels at, especially Cd 0, which led to an increase in the content of chlorophyll in the leaves reaching 37.81 mg.g<sup>-1</sup>, while the highest levels of Cd resulted in lower chlorophyll content, at 24.56 mg.g<sup>-1</sup>, which is clear in Figure 8, agreeing with Drązkiewicz et al.,<sup>8</sup> John et al.,<sup>12</sup> and Slaski et al.<sup>27</sup> This inverse correlation between chlorophyll content and Cd concentration might be caused by Cd inhibiting the enzymes that contribute to the process of creating chlorophyll.<sup>14</sup>

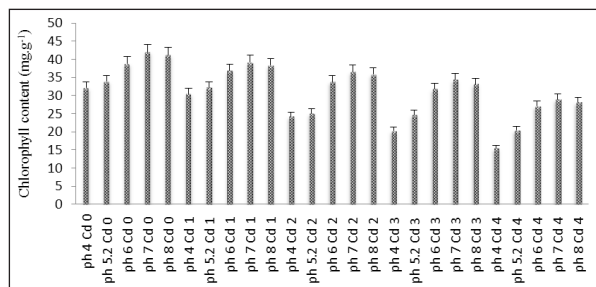
Increased acidity of the soil is regarded as a main factor that controls the availability of Cd.<sup>13,25</sup> This might agree with our results reported in Fig. 9, where the effect of interaction between the two study factors pH 4 and Cd 4 substantially affected the decrease of the chlorophyll content in leaves, where the rate reached 15.79 mg.g<sup>-1</sup> due to the influence of low soil pH to the absorption of Cd, consequently leading to the inhibiting of the functions of some necessary enzymes in the process of creating chlorophyll, and from another side, the low soil pH prevented the absorption of necessary nutrients by the plants, such as N. This is evident in the interaction between pH 7 and Cd 0, which resulted in the highest content of chlorophyll in leaves, reaching 42.32 mg.g<sup>-1</sup>. This increase resulted in the prevention of absorption of Cd at high soil pH; this is confirmed by Nyaenya.<sup>19</sup>



**Figure 7. Effect of Soil pH Levels on Chlorophyll Content (mg.g<sup>-1</sup>)**



**Figure 8. Effect of Cadmium Levels on Chlorophyll Content (mg.g<sup>-1</sup>)**



**Figure 9. Effect of Interaction between Soil pH and Cadmium Levels Chlorophyll Content (mg.g<sup>-1</sup>)**

## Conclusion

Cadmium contamination is one of the increasing concerns worldwide, thereby various scientists proposed different strategies to overcome this environmental problem. The present study was conducted to know the effect of pH of soil with cadmium on nitrogen, phosphorus and chlorophyll contents in the corn plant. To achieve this, experiments were conducted at the Institute of Sustainable Agrotechnology (INSAT), University Malaysia Perlis in Sungai Chuchuh, Perlis, Malaysia, to determine the levels of soil acidity that increase and the levels that reduce the nitrogen, phosphorus and chlorophyll contents in the plant. Whereas the soil pH 7 led to significant effect in increase the nitrogen and chlorophyll contents in the corn, the level of pH 7 gave highest contents for nitrogen and chlorophyll compared with the rest of other levels. While the decrease of soil pH led to significant effect to increase phosphorus content in the corn, the level of pH 5.2 gave the highest content. Also the increase concentration of cadmium led to significant decreasing in nitrogen, phosphorus and chlorophyll contents. As for the interactions between soil pH and cadmium, interaction between pH 4 and Cd 4 led to decrease of the nitrogen and chlorophyll contents in the corn, while the interaction between pH 8 and Cd 4 led to decrease in phosphorus contents in the corn.

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