

Toxic Effects of Common Inorganic Fertilizers on Mortality, Hemoglobin Levels, and Behavior in *Lumbricus terrestris*

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ABSTRACT

The current research seeks to determine the impact of chemical fertilizers employed in Iraqi agriculture on soil-dwelling organisms. Investigating the impact of fertilizers on organisms is essential for establishing lethal and sub-lethal tolerance limits and determining lethal concentrations. The research aims to identify the impact of chemical fertilizers utilized in Iraqi farms on soil-dwelling organisms, prompted by a deficiency of comprehensive studies in this area. Among the significant organisms with distinct roles in soil ecosystems, annelids (earthworms) were selected to assess these effects. They are crucial organisms due to their contributions to soil health and their sensitivity to toxic chemicals, serving as vital indicators for the presence of pollutants in the soil. Samples of adult earthworms exhibiting saddle structures were collected and placed in plastic containers filled with soil to a depth of 15 cm. Earthworms were subjected to three types of fertilizers (NPK) comprising phosphorus, potassium, and nitrogen sourced from local markets, commonly utilized in gardens and farms, revealing a significant effect of fertilizers on earthworms. Mortality rates increased notably with higher fertilizer concentrations and longer treatment durations. Additionally, hemoglobin levels in earthworms rose, indicating environmental stress, prompting the worms to elevate their hemoglobin levels.

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1. INTRODUCTION

Earthworms play a crucial role in soil well-being and have been recognized since ancient times; Aristotle referred to them as the "intestines of the earth" due to their function in decomposing plant material and converting it into simpler organic and inorganic compounds. Charles Darwin studied earthworms for over 40 years, referring to them as small, organized creatures. He emphasized their significance in enhancing plant production through soil aeration and the provision of essential organic and inorganic materials for plant growth. Additionally, he noted their role as a food source for fish and birds [1]. Earthworms are regarded as environmental engineers due to their behavior of burrowing and incorporating leaves and fertilizers into the soil, thereby influencing its physical properties, nutrient circulation, and plant growth [2] [3]. These worms are distributed worldwide. Certain species inhabit specific regions and are referred to as native species. Europe is widely regarded as the native home of worms, from which they spread globally. They were introduced to America in the sixteenth and seventeenth centuries by travelers who brought European plants along with the soil-dwelling worms [3]. Earthworms prefer to live in moist soils rich in organic materials [4]. Some species of these worms live at different levels within the soil layers or on its surface. Some live in pieces of rotting wood or in the axillary of tree trunks (the upper angle between the branch and the trunk) or near lakes, rivers, springs and ponds. They are abundant in mineral soils containing carbon and tropical soils that contain large amounts of humus [5].

Several factors influence earthworm density, including humidity, temperature, pH level, tillage practices, application of fertilizers and pesticides, soil contamination with heavy metals, presence of predators, soil texture, and the cultivated crop. In recent years, farmers' use of chemical fertilizers to fertilize their crops has increased due to the need to secure food sources in large quantities, which leads to agricultural products characterized by their bulk and excessive early production. Fertilizers are natural or synthetic substances that supply critical nutrients to plants for their growth, development, and enhanced yield. Fertilizers are categorized into organic (natural) fertilizers, comprising animal and plant waste, and chemical (industrial) fertilizers, produced from mineral and chemical substances in specialized factories. The latter are further divided into simple fertilizers containing a single element, such as nitrogen and phosphorus (NP), nitrogen and potassium (NK), or a combination of nitrogen, phosphorus, and potassium (NPK) [6].

One of the most important uses and advantages of natural fertilizers is that they improve the composition and structure of the soil and its ability to absorb moisture, which is rarely toxic or harmful to plants or the environment. As for chemical fertilizers are rapidly decomposed; therefore, their effectiveness is immediate and they contain known proportions of added elements compared to natural fertilizers. Also, exceeding the use of added quantities beyond specific proportions, which often happens through repeated, unstudied and random additions, will negatively affect the ecosystem's living components; [7]. Nitrogen fertilization is one of the most critical pollutants of water, soil, and air. As for phosphate fertilizers, their long-term use increases pollution with residues of some elements such as lead, arsenic, and cadmium. It also affects the change in the chemical and physical properties of the soil, such as the pH, which clearly affects living organisms that inhabit the soil. Moreover, changing soil acidity produces intricate consequences, as the uptake of certain elements increases while the absorption of others decreases at specific levels of acidity or alkalinity. Moreover, altering the soil's acidity may influence the transformation of certain elements from non-toxic to poisonous compounds. Furthermore, the fertilizers employed may leak, adversely impacting the type and quality of groundwater, posing risks to living organisms [7] [8].

From local studies that included the distribution of earthworms [9] [10] [11] They found, Earthworms can be found in a variety of agricultural fields and have a wide range of physical and chemical characteristics; they are also thought to be a good indicator for detecting the effects of such environmental change, Earthworms derive energy to carry out various vital activities through their consumption of food, plant leaves, animal dung, fungi, bacteria, and the remains of dead animals that inhabit the soil, And play an important role in the soil biota community; they are responsible for decomposing, aerating, recycling nutrients, and increasing agricultural products.

As for studies on toxic earthworms [12] Included Efficiency of earthworm in bioremediation of heavy elements and recycling of organic waste, study dealt with, the bioremediation of heavy metals using two species worms, *Eisenia fetida* and *Lumbricus terrestris*, The results showed significant differences in the carbon to nitrogen ratio in the organic fertilizer for worms, as it was the highest in *Lumbricus terrestris* with a ratio of 21.987. The results also showed significant differences in the quality of organic waste, as the highest carbon to nitrogen ratio was measured in *Lumbricus terrestris* reached 20.72. also measured Concentration of heavy metals in cow and buffalo manure The results showed significant differences for the element chromium, and the highest concentration in *Eisenia fetida* manure reached 16.316 parts per million. While [13], studied The histological change caused by pollutants is considered as a sensitive tool to determine the direct effect of those chemical pollutants on the test organism. This study was done to determine changes caused by pesticide methiocarb in earthworms. While they studied [12] Bioremediation of Petroleum-Contaminated Soil Using Earthworms: A Study on Hydrocarbon Reduction in Southern Iraq, the statistical results showed that the earthworms significantly reduced total petroleum hydrocarbons (TPH), it was concluded that vermiremediation using earthworms was very effective in improving soil contaminated with crude oil and worked to reduce the level of contamination with total petroleum hydrocarbons, and it also led to soil neutralization by reducing the pH. Therefore, there are no local studies on the effect of fertilizers on soil life, especially earthworms, and this is where the idea of the current research came from.

In light of insufficient extensive studies, the current research seeks to determine the impact of chemical fertilizers employed in Iraqi agriculture on soil-dwelling organisms. Among the most significant creatures with a specific role in the soil, annelids were selected to demonstrate these effects. Earthworms are essential living organisms because of their role in the soil are sensitive to toxic chemicals and have important biological indicators of the presence of pollutants in the soil.

2. MATERIALS AND METHODS

Adult earthworm samples containing saddle clitellum were collected and placed in plastic containers filled with soil to a height of 15 cm [14]. The soil characteristics were as follows: pH 7.5, salinity 0.15, organic matter 2.82, soil moisture 40%, calcium carbonate (CaCO₃) 4.89%, carbon-to-nitrogen ratio (C: N) and total nitrogen, total sodium 0.02%, and total phosphorus 5.75%. The soil texture was clay 9.33%, silt 53.53%, and sand 37.14%. The samples were kept under laboratory conditions at 25–30°C for a week to acclimate before the experiment was conducted. Earthworms were exposed to three types of fertilizers (NPK): phosphorus, potassium, and nitrogen obtained from local markets (used locally in gardens and farms) at concentrations of (0.5, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 grams).

The treatments were repeated three times, and each replicate included 50 worms. The number of fatalities was calculated after 24 hours, 48 hours, and one week from the treatment, and the lethal concentrations and the median lethal concentration (LD₅₀) were calculated according to the [15]. The statistical program was used to analyse the data, and analysis of variance and the least significant difference test were employed to determine the essential differences between the means of the transactions at a significance level of 0.05. To assess fertilizers' effect on worms' biological indicators, the measurement of hemoglobin levels was chosen according to the method [16]. Additionally, the worms' activity, movement, and shapes were observed, recorded, and photographed.

3. RESULTS AND DISCUSSION

The study of the effect of fertilizers on organisms is necessary to determine the limits of lethal or sub-lethal tolerance and the concentration of the killer. The current study showed an apparent effect of the fertilizers used on earthworms, as deaths increased significantly with increasing fertilizer concentrations and treatment duration. There was also an evident increase in the hemoglobin levels of earthworms, which is a vital indicator of environmental stress. Therefore, worms resort to raising hemoglobin levels. These organisms pick up chemicals from soil and water, either by swallowing soil or through the skin, representing the surface for exchanging respiratory gases. Earthworms do not have a specialized respiratory system, as oxygen diffuses through the tissues directly into the blood, is absorbed by hemoglobin (Hb), and is distributed throughout the body. Through follow-up, it was found that fertilizers have a clear effect on the movement and activity of worms, and high concentrations showed a clear impact on the colors of worms (Figure 1).

3.1. The Effect of NPK Fertilizers on Soil Properties

The results of the current experiment showed that fertilizers have a clear effect on soil properties, as fertilizers had a clear effect on PH values, as the values decreased and tended towards acidity in the soil treated with phosphorus and potassium fertilizers, reaching 5.5 and 6.5 in each of them, while the pH increased towards basicity in the soil treated with nitrogen, reaching pH 8.

Fertilizers significantly influenced the C/N value, a critical indicator of the quality of organic matter added to the soil. According to international fertilization standards, the optimal C/N ratio should fall within the range of 20-30. [17] noted that a high percentage of this indicator impacts decomposition processes, resulting in soil organisms not benefiting from organic matter regarding element availability until after an extended period. Conversely, a low C/N ratio results in nitrogen loss as NH₄. Organic matter is crucial in the soil carbon cycle, primarily composed of carbon (C), hydrogen (H), and oxygen (O), along with contributions from nitrogen (N), phosphorus (P), and sulphur (S). After the decomposition of organic matter, the soil content of carbon and other elements changes, as the relationship is negative between soil carbon and its content of the primary nutrients that make up organic matter. Soils with low organic carbon content have high nitrogen, phosphorus, and sulfur content, while the opposite is true for soils with higher organic carbon content. This varies depending on the ratio of carbon to nitrogen in organic matter [18]. Using fertilizers also had an apparent effect on the values of organic matter, the percentage of phosphorus and potassium, and an apparent increase in salinity rates, as shown in Table (1).

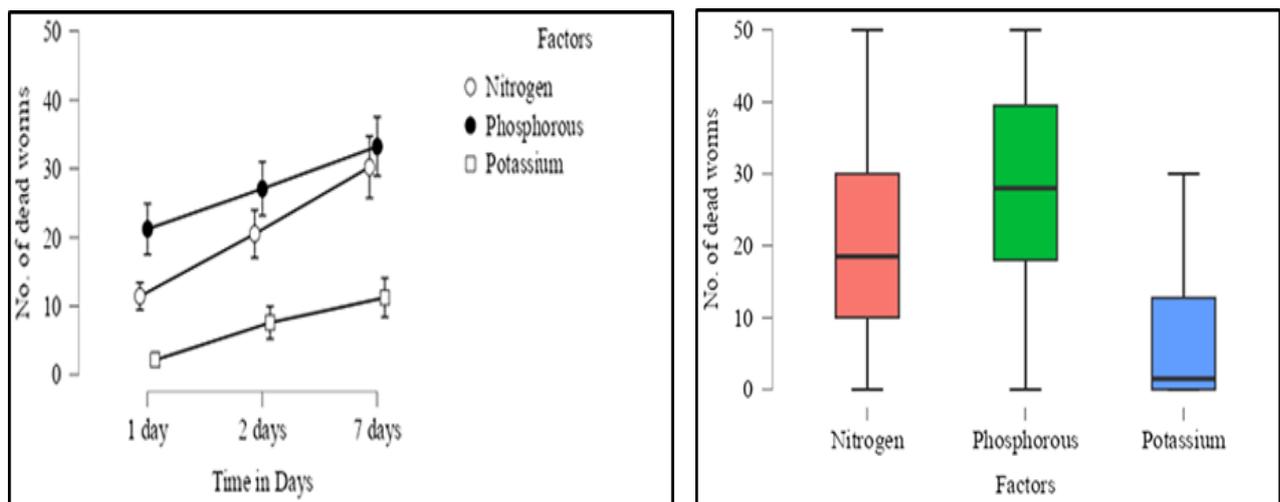
Table 1. Some physical and chemical properties of soil treated with NPK fertilizer

Fertilizers\Properties	TM	ph	salinity	Soil Moisture	Organic Matter	Calcium	CaCO ₃	C: N ratio	total n	k	p
Control	25	7.5	0.15	27.24	2.82	167	4.89%	20.4	0.019%	139	5.79
Phosphorus	25	5.5	0.4	27.23	2.7	177	6.9	50.13	0.02	140	8.7
Potassium	25	6.5	0.2	27.24	2.8	178	5.5	54.2	0.03	199	6.8
Nitrogen	25	8	0.3	27.23	2.9	189	5.9	48.2	0.05	145	5.4

3.2. The Effect of Potassium Fertilizer K on Earthworm *Lumbricus terrestris*

Through the results of the study and statistical analysis; it is clear that potassium fertilizer, K, was less effective and toxic during the first day compared to phosphorus and nitrogen when the mortality rates were clearly low, especially during the 24 hours (Figure1), after treatment, as the concentrations (0.5, 1, 1.5, 2, 2.5, 3) g did not record any mortality, while mortality began within a day of exposure at concentrations (4.4.5, 5, 6, 7, 8). Furthermore, there was no effect of potassium on hemoglobin levels (Figure1) during 24 hours of exposure, while after 48 hours of exposure to potassium fertilizer (Figure1), it was found that mortality began at a concentration of 3.5 g, however it was low compared to phosphorus and nitrogen fertilizers, as mortality rates ranged between 5 worms as the lowest value at a concentration and 25. The highest value was recorded at a concentration of 8 g, and the worms began to show vital reactions as the hemoglobin began to rise slightly. After a week of treatment (Figure 1) with potassium fertilizer, the number of deaths increased to reach the lowest value of 1 worm at a concentration of 1.5 g and the highest value of deaths reached 30 at a concentration of 8 g. A slight increase in hemoglobin levels was also evident to reach the highest value of 0.5 mg.

Figures (1) and (2) show that the average values of potassium fertilizer concentrations that are lethal to half the number (LD 50) over 24, 48 hours and a week of exposure to earthworms reached 7.5, 10.5, 7.5 and 6.7 respectively. Potassium fertilizer did not record a 100% killing rate.

Figure 1. Effect of different fertilizers on the number of *Lumbricus terrestris* deaths

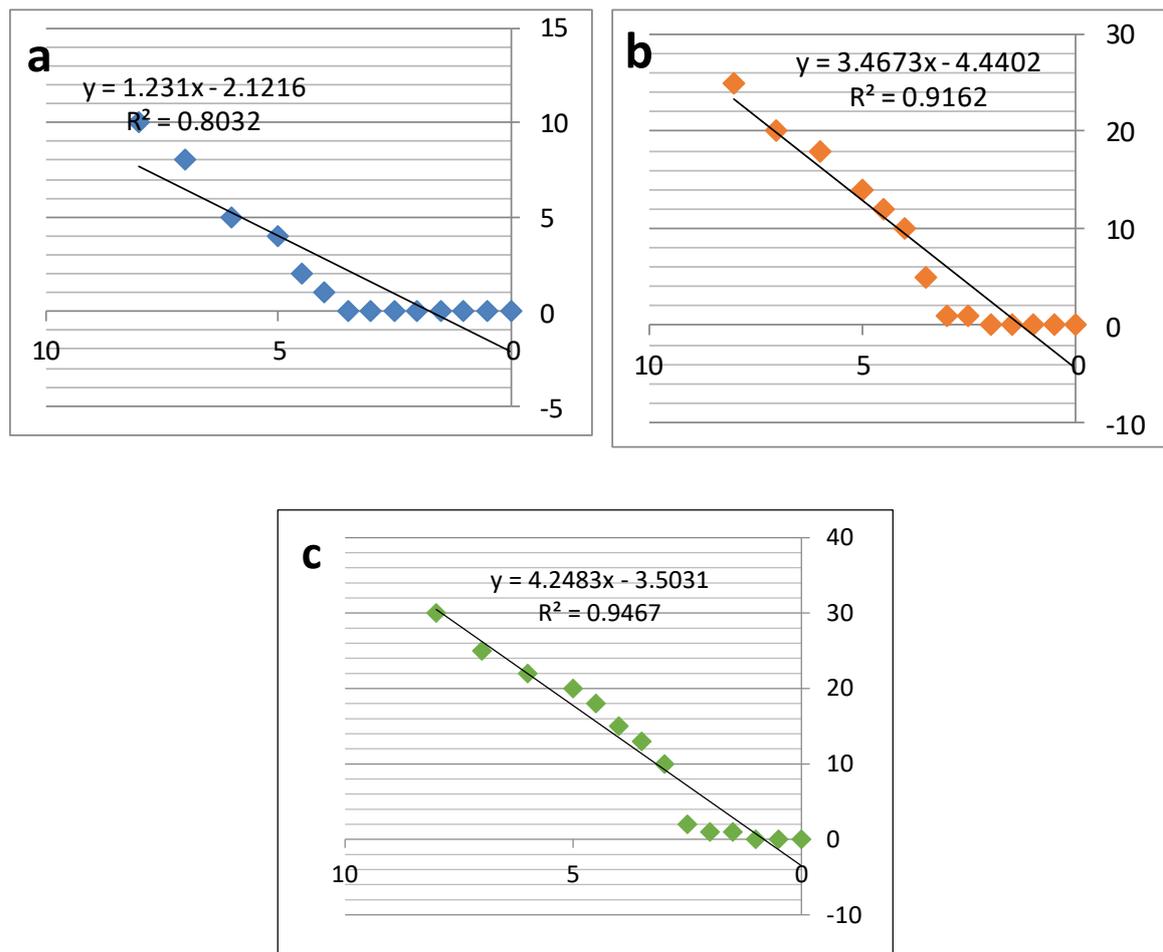


Figure 2. Effect of potassium fertilizer on mortality rates after (a-24, b-48 and c- 1 week) of *Lumbricus terrestris* earthworms

3.3. Effect of Nitrogen Fertilizer N on *Lumbricus terrestris*

As for the results of treating earthworms with nitrogen fertilizer, the results of the current study showed that after 24 hours of treatment, deaths began to appear at a concentration of 1 g, reaching 5 worms and represented the lowest value, while the highest value of deaths was recorded at a concentration of 8 g, reaching 25 worms. Also, no effect was observed for all concentrations of nitrogen fertilizer on hemoglobin levels, as they were constant at 0.19 g/dl. While the mortality rates increased after 48 hours, and the increase was clear with increasing concentrations, as it reached the highest value of deaths during the highest concentration of 8 g. Hemoglobin levels also began to rise, meaning that worms began to be affected by the presence of fertilizer in the soil in which they live, which led to raising hemoglobin levels in their bodies, as they reached 0.4 mg at concentrations of 7 and 8 g. Figures (1) and (2) indicate that the average lethal amounts of potassium fertilizer for 50% of earthworms (LD50) after 24 hours, 48 hours, and one week of exposure were 7.4, 8.03, and 2.33, respectively. In the case of nitrogen fertilizer, the LD 50 was determined to be 2.399 g after one week of treatment, but the quantity resulting in 100% fatality was identified as 7.15 g. After one week, the impact of nitrogen fertilizer was evident, with fatalities reported at a concentration of 1 g, totaling 15 worms. Concentrations of 7 and 8 exhibited the highest mortality rates, resulting in the death of all worms in the experiment, so achieving a mortality rate of 100%. The worms exhibited distinct biological responses by elevating haemoglobin levels with increasing concentrations, achieving 0.7 and 0.8 mg, which were significantly higher than the control group's normal levels of 0.19 mg. Figures (1) and (2) illustrate that the average lethal concentrations of potassium fertilizer (LD 50) after 24 hours, 48 hours, and one week of earthworm exposure were 1.75, 7.5, and 1.8, respectively. Potassium fertilizer did not record a 100% killing rate. Nitrogen fertilizers are converted into ammonia, which activates the soil organisms responsible for the nitrification process that converts ammonia into nitrates. Urea is one of the compounds produced from ammonia and CO₂. When fertilizing with urea, urea is decomposed by soil microorganisms into ammonia and CO₂.

The most important characteristics of urea are its content of 46% nitrogen and 1% biuret. When the biuret concentration exceeds 3%, it is considered toxic to plants. It affects seed germination, plant tissues, and protein synthesis. Additionally, increased rates of urea decomposition lead to ammonia formation, which raises the soil pH value. This aligns with the current study's results, as pH values increased significantly, leading to ammonia volatilization. Nitrates are considered dangerous pollutants for human health because nitrates themselves are not toxic, the real danger of nitrate compounds lies in the fact that part of them is reduced to the toxic nitrite ion (NO_2) in the digestive tract (stomach and intestines). The toxicity of nitrite comes from its absorption into the blood, leading to the oxidation of iron in hemoglobin in the Fe^{+3} state, causing red hemoglobin to turn dark. This condition results in a serious disease, which explains the current study's findings and the noticeable change in hemoglobin levels of earthworm.

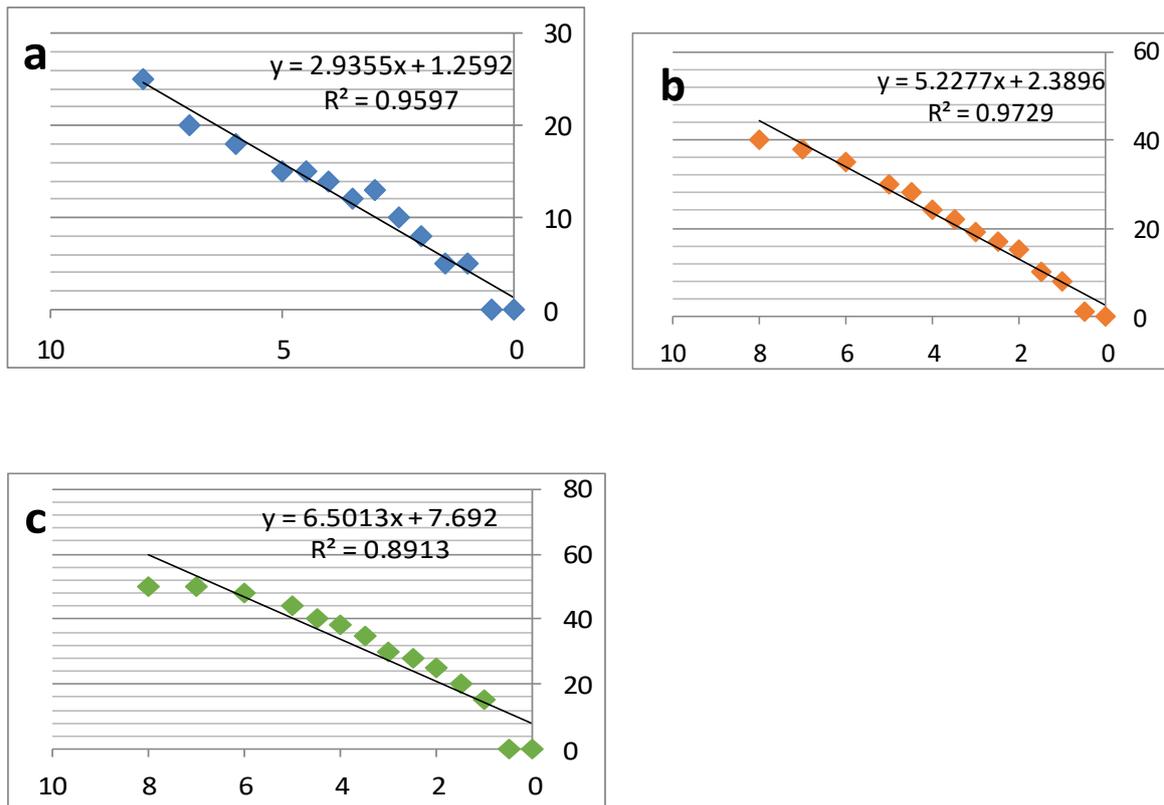


Figure 3. Effect of nitrogen on mortality rates of *Lumbricus terrestris* after (a-24 ,b- 48 and c-1 week)

3.4. The Effect of Phosphorus Fertilizer P on *Lumbricus terrestris*

Phosphorus fertilizer was the most effective and the most toxic, as the results of the current experiment showed that phosphorus fertilizer caused the death of 8 worms at a concentration of 1 g and after 24 hours of treatment, and the deaths began to gradually increase with increasing the concentrations, reaching 45 worms out of a total of 50 ones at a concentration of 8 g. It also became clear that the worms were exposed to stress due to treatment with phosphorus fertilizer, as hemoglobin levels began to rise from the first day of treatment, reaching 0.3 mg at high concentrations of 7 and 8 g. After 48 hours of treatment, deaths began to appear at a concentration of 0.5 g to reach 50 worms, i.e. the death of all worms in the experiment at a concentration of 8 g. Hemoglobin levels also increased significantly with increasing the concentrations to reach 0.5 mg at the high concentrations of (7 and 8) g. These percentages are high compared to the control group worms, as the normal hemoglobin levels were 0.18 mg. Phosphorus fertilizer had the most toxic and lethal effect after a week of treatment, as it recorded higher mortality rates than potassium and nitrogen at the same concentrations, as the concentration of 1 g recorded mortality rates that reached 18 worms, and mortality continued to increase with increasing concentrations to reach 100% at concentrations (6, 7, and 8) g. Hemoglobin levels also reached the highest rate during the period and concentrations of the different fertilizers used, as it reached 0.9 mg at concentrations (5, 6, 7, and 8) g.

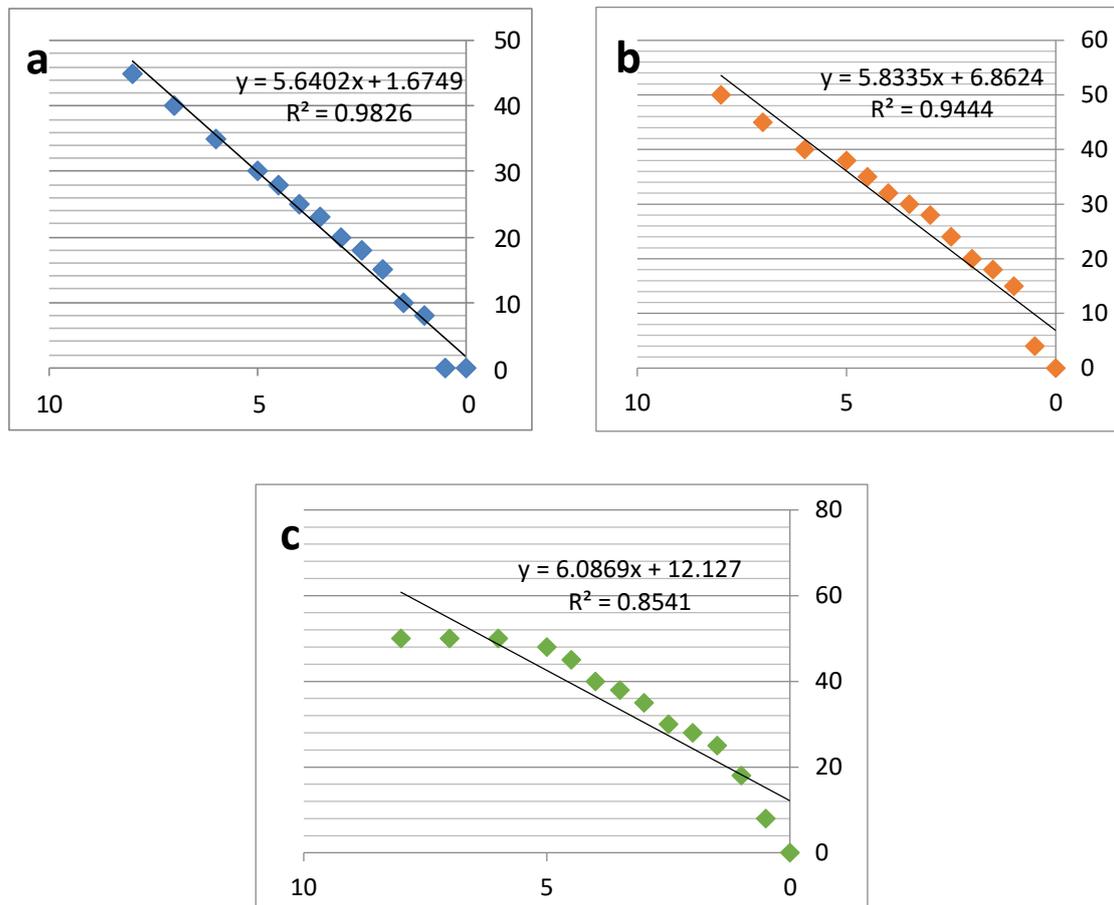


Figure 4. Effect of phosphorus fertilizer on mortality of *Lumbricus terrestris* rates after (a-24, b-48 and c-1 week)

3.5. The Effect of Fertilizers on Hemoglobin Levels and Some Vital Indicators of *Lumbricus terrestris*

Based on the results of the current study, the impact of using fertilizers on earthworms is evident, as the earthworms showed a clear sensitivity through increased mortality rates and higher hemoglobin levels. This is consistent with the study by [19], which found that the exposure of earthworms to heavy metals caused an increase in hemoglobin levels. This is a vital indicator which organisms resort to in case of environmental stress or the presence of pollutants in their environment, similar to non-biting mosquito larvae that increase their respiratory pigment levels when exposed to pollutants [19].

The results of the current study are consistent with many other studies, as they indicated that the most important factors affecting the activity and density of earthworms are using fertilizers and pesticides due to their toxicity [20]. They accumulate in their bodies because the nature of earthworm feeding involves consuming soil and plant leaves, and in turn, all pollutants enter their bodies or are affected by the presence of fertilizers as they alter soil properties, especially changing pH values. This is considered one of the most significant factors affecting the activity and survival of earthworms. Additionally, fertilizers have clearly impacted the immune system, as earthworms possess an immune system represented by coelomic cells [21]. The immunity in earthworms takes two forms: one is cellular, which includes coelomic cells located in the coelomic fluid, surrounded by a squamous epithelial tissue situated between the intestines and the muscular body wall. The other type is humoral, which is important in toxicity-related experiments. [22] mentioned that the rate of coelomocytes varies between species, with the highest rate in the species *L. terrestris* compared to the species *Octolasion tyrtaeum* and *E. fetida*. [23] specified the impact of environmental pollutants on the coelomic cells of worms, where their inflammatory function is significantly affected. These metals also influence the vitality of the coelomic cells, and lead to the fragmentation of the coelomic cells within the coelomic cavity. According to [24], the impact of environmental pollutants on the survival, reproduction, and coelomic cells of the species *Allolobophora chlorotica* was studied. It was found that adults die at a high rate, reproduction is inhibited, and there is a weakness in the coelomic cells, which in turn weakens the immune functions of this species. Additionally, the discussion results showed that fertilizers had a clear effect on the weights of the worms (Figure 6), as they decreased to the lowest weight of 1.9 at a concentration of 8 with phosphorus treatment, while with potassium, it reached 3 at a concentration of 8, and with nitrogen,

it reached 2 at a concentration of 8, clearly after treatment. It also led to changes in the colors and movements of the worms (Figure 1).

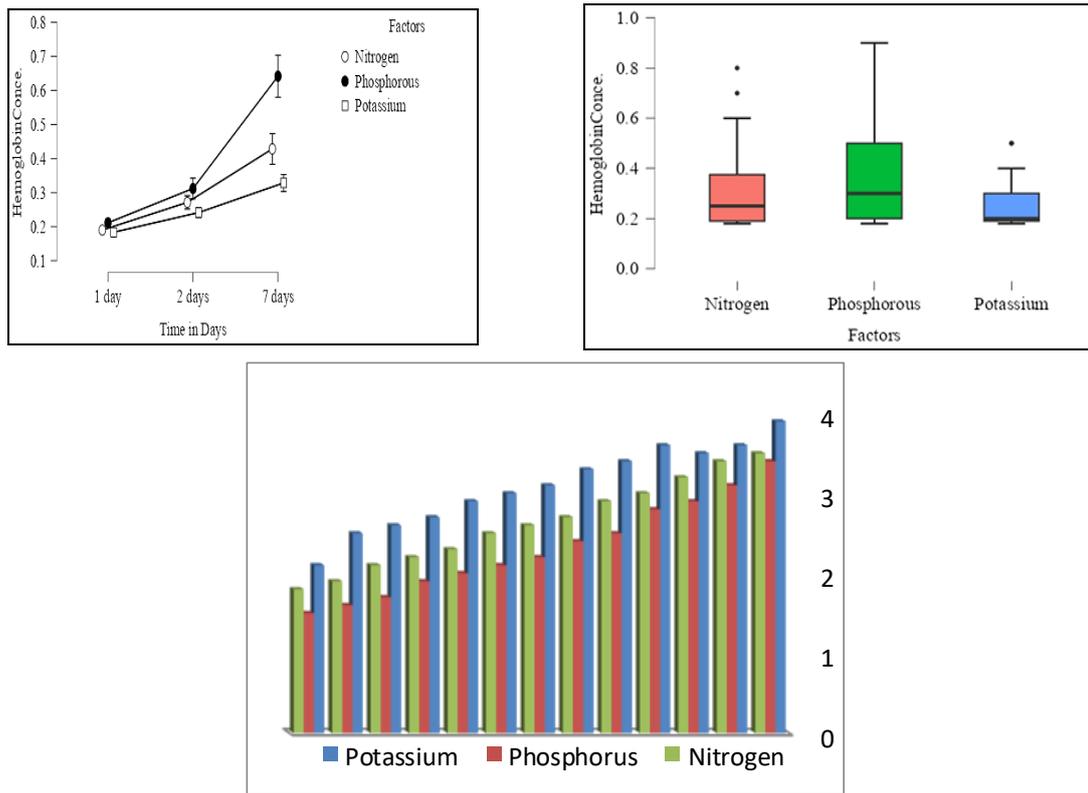


Figure 5. Effect of different fertilizers on Hemoglobin levels of *Lumbricus terrestris*

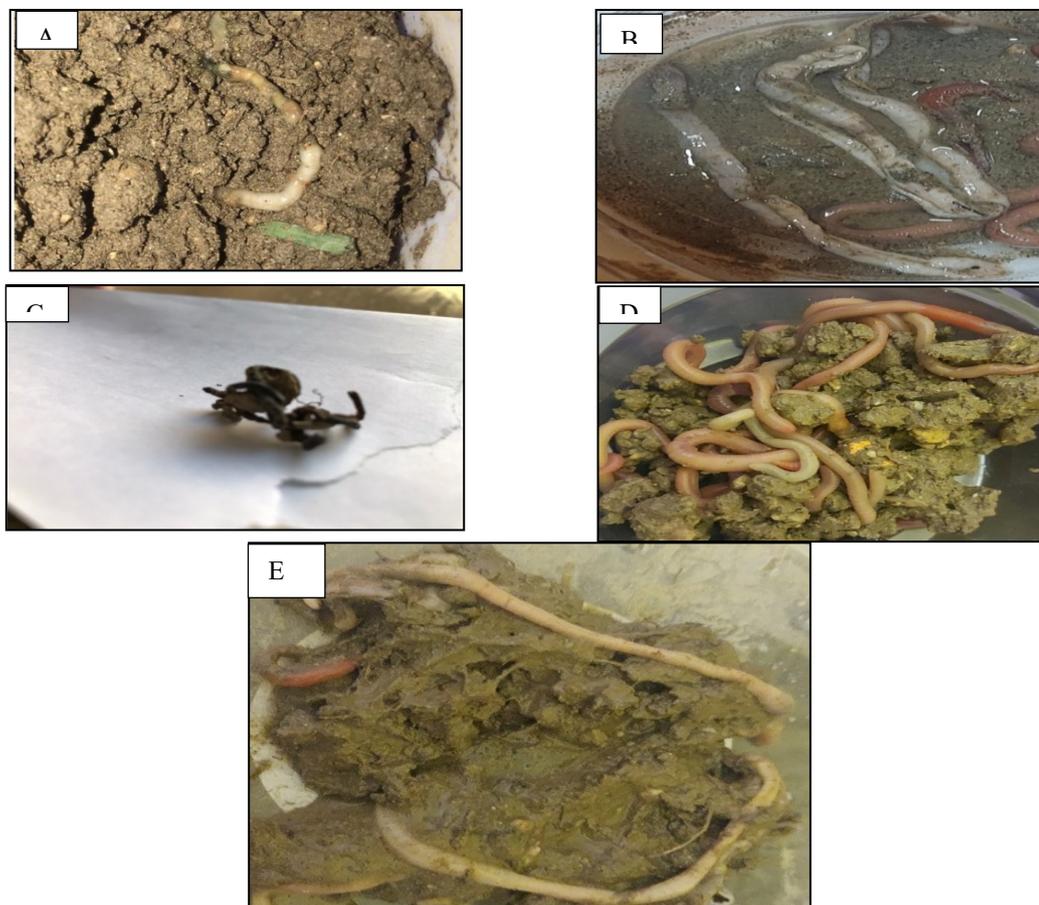


Figure 6. Earthworm before treatment (D) and after treatment (A, B, C, E deformities)

4. CONCLUSION

Earthworms can be found in a variety of agricultural fields and have a wide range of physical and chemical characteristics; they are also thought to be a good indicator for detecting the effects of such environmental change, Earthworms were subjected to three types of fertilizers (NPK) comprising phosphorus, potassium, and nitrogen sourced from local markets, commonly utilized in gardens and farms, revealing a significant effect of fertilizers on earthworms. Mortality rates increased notably with higher fertilizer concentrations and longer treatment durations. Additionally, hemoglobin levels in earthworms rose, indicating environmental stress, prompting the worms to elevate their hemoglobin levels, It also led to changes in the colors and movements of the worms.

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