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Research Article

Study of the Effect of Mercury Salt on the Presence of Annelida (*Lumbricus terrestris*) in Soil and the Histological Changes on the Skin Tissue

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The earthworm is the most beneficial organism for assessing metallic contamination due to the fact that critical high-quality correlations have been determined between earthworm steel concentrations and soil cadmium, copper, lead, zinc, and mercury concentrations. The mercury compounds are very poisonous for most organisms. Here, we investigated the ability of earthworms (*Lumbricus terrestris*) to $HgCl_2$ (below laboratory conditions). A study of the effect of five different salt concentrations of mercury chloride ($HgCl_2$) 1 ppm, 5 ppm, 10 ppm, 50 ppm, and 100 ppm was studied on worm survival and calculation of the concentration required to destroy half the number of worms (LC50). The results showed that there is an inverse relationship between these salt concentrations and the time required to perish 100% of worms. It was found that the concentration of 100 ppm is the lethal concentration of half the number (LC50) of the earthworm (*Lumbricus terrestris*). The effects of the salt concentrations used confirmed their effect on the experimental worm weights as well, so the concentration of 50 ppm was the most influential on earthworm weights of the type of *Lumbricus terrestris*, as it caused a reduction 12% of worm weights with a significant difference, while the concentrations 1 ppm and 100 ppm were the least effective, as they caused the worms to decrease by only (5%), while the remaining two concentrations 5 ppm and 10 ppm caused their effect to decrease by 7% only. Thus, it can be concluded that lower worm weights have nothing to do with the lethal concentrations of the heavy metal salt ($HgCl_2$). The histological study also showed that low concentrations of mercury chloride salt have less effect on earthworms' skin tissue compared to higher concentrations.

1. Introduction

Potentially toxic factors in soil include metals and minerals, including cadmium (Cd), mercury (Hg), lead (Pb), or micronutrients, including chromium (Cr), selenium (Se), copper (Cu), and zinc (Zn), which can also appear toxic when concentrations exceed tolerances [1, 2]. Since now all factors are no longer poisonous, the term "poisonous elements" have gained greater scientific acceptance in the literature as a more comprehensive and relevant term than poisonous metals or metalloids [3]. Toxic elements spread naturally on the Earth and in acceptable concentrations [4]. However, degrees of probably poisonous factors in soils have multiplied dramatically global because of the commercial revolution because of big human activities [1, 5]. Fertilization with pesticides (agricultural) is a potential human source of potentially toxic elements in the soil [6]. So, mercury (Hg) is a natural metal and is found in the environment by many natural and anthropogenic processes [7, 8]. Mercury is especially solid withinside the ecosystem

and might unfold over the complete globe earlier than returning to the Earth's surface. In the soil, mercury is largely static, accumulating within the apex layer, specifically via means of binding to natural matter, specifically to thiol groups [9].

More than 90% of the soil invertebrate biomass is made up of earthworms, which usually play important roles in many soil formation processes [10].

It also serves as a great food source for many higher organisms, such as birds, and is used to feed fish of commercial importance because it contains a high concentration of proteins rich in essential amino acids, which are used as bait for fishing [7]. It is also used as a biomarker in environments as it is five-pointed for assessing toxicity in aquatic and land environments, as it is considered as a fertilizer for aquatic plants through their excrement and decomposition after death [11].

Recently, antihelminthic treatment has attracted significant scientific interest as an expanded technology for the biological treatment of potentially toxic elements in contaminated soils [12–16]. Nonetheless, earthworm abundance and network shape are dependable signs to evaluate soil infection and also can repair infected soil through bioaccumulation [17–20]. For example, greater probably poisonous factor elimination in earthworm-assisted treatment of municipal wastewater, manure, and different probably poisonous factors-wealthy natural wastes had been reported [21–24]. However, the systematic evaluation of earthworms' ability to potentially toxify elements and treat infected soils is limited, and only some opinions have been published on the use of earthworms to treat infected soils [25, 26]. Even so, earthworms offer a very good ability to treat toxic agents in infested soils but are rarely evaluated [19]. Earthworms are crucial biomarkers of soil contamination. The bioavailability of mercury relies upon the species, which in flip determines the toxicity, transport, and time for the metal withinside the environment [7]. Many researchers have indicated the ability of earthworms to take in heavy elements and accumulate them in their tissues, such as mercury, lead, cadmium, gold, and selenium [8]. It was found that these pollutants have the ability to affect earthworm communities in terms of killing them or reducing their numbers and presence [9].

Heavy metals did not affect worms only, but there is an effect on many aquatic organisms such as fish, soft shellfish, and crustaceans [10].

In view of the pollution of soil in Anbar Governorate as a result of military operations and war remnants whose remnants have leaked into water and soil because they contain heavy elements, their toxic concentrations, and their danger to the environment, and due to the ability of oligochaetes to accumulate in their tissues, the idea of the current study resulted in which the high-capacity worm accumulates elemental mercury in the tissue to study the histological changes in cells.

2. Materials and Methods

Experimental earthworms were collected from a home garden in which no chemicals or pesticides were used. They were isolated from the mud by placing the mixture in a sieve, washed well with water, and brought to the laboratory.

Active adult worms were isolated and classified, and the type *Lumbricus terrestris* was selected for the experimental study, taking into account equal lengths and sizes as much as possible. These worms were placed in plastic basins containing an appropriate amount of sandy soil mixed with clay with a pH of 7.6 and left under laboratory conditions (20°C temperature and 60–70% humidity) for a period of fourteen (14) days in order to acclimatize, observe their movement and activity, and isolate the dead worms, as well as the inactive, taking into account the continuous moistening of the soil to ensure that the worms do not dry out and die, and then a process of isolating the adult worms was conducted from both types in separate ponds.

In each experiment, 36 adult and active worms were used, taking into account equal lengths and sizes as much as possible. The worms were divided into six (6) groups, each group includes six (6) worms, with five replicates for each concentration, including the control group. They were placed in small plastic ponds containing an equal amount of moist soil, and then each group was offered 1, 5, 10, 50, and 100 ppm of mercury chloride salt.

The groups were examined after 24, 48, and 72 hours to calculate the dead worms and determine the lethal concentration of half the number (LC50). The experimental groups were left for 21 days with a daily examination, and by light pricking using forceps and under an anatomical microscope, it was confirmed that the worms were dead or still alive.

The phenotypic and histological changes that occurred in the worms as a result of exposure to different salt concentrations of mercury chloride were also studied.

2.1. Histological Technique

(1) Samples from the skin of worms were collected for the purpose of the histological study: Fixation: Each tissue sample is usually cut into small pieces of 2–3 cm in length prior to fixation to facilitate penetration of the fixative and tissue preservation. The best fixative for routine light microscopy is an isotonic solution of 10% Bowen's solution for 24 h.

(2) Embedding: The process of paraffin embedding, or tissue impregnation, is ordinarily preceded by two main steps:

   (A) Dehydration: Water is firstly extracted from the shrapnel to be immersed with the aid of bathing successively in a graded combination of ethanol and water (generally 70% to 100% ethanol) for 1 h for each concentration, while in a day to obtain absolute ethanol

   (B) Clearing: The ethanol is then changed with a miscible solvent with the embedding medium; the solvent used is generally xylene for one hour. The tissue is positioned in melting paraffin in the oven, normally at 58–60°

(C) The solid masses containing the tissue were then transferred to a microtome and sliced to 5 μm thickness. Sections float on water and are transferred to glass slides to be stained.

(3) Hematoxylin and Eosin stain Procedure: the tissue is dewaxed by xylene. The slides were washed in three changes of absolute alcohol, then 95% alcohol, and70% alcohol, 5 minutes before each step. The slides were washed in water for 5 minutes and then stained with hematoxylin for 5 minutes. The slides had been washed in water for three minutes. The slides had been then located in eosin for 10–15 seconds. The slides were washed in water for 2–3 minutes. The sections were then dehydrated in 70%, 80%, and 95% alcohol for a few seconds to each step, followed by two changes of absolute alcohol for 5 minutes each. The final step was to put the slides in xylene for 5 miminutes.

(4) Mounting: the sections were mounted by DPX, then they were covered with cover slides and left at room temperature to dry.

2.2. Statistical Analysis. Statistical analysis with differences between the two groups was performed by one-way analysis of variance (ANOVA), and Dunkin' test by Costas software (Monterey, CA, USA). The experimental data were presented as (mean ± SE) with a significant ($P \leq 0.05$).

3. Results and Discussion

The results of the effect of the different salt concentrations of mercury chloride (HgCl2) on the survival of the worms showed that there is an inverse relationship between the salt concentrations of mercury chloride and the time required for the perdition of 100% of the individuals.

Regarding the earthworms *Lumbricus terrestris*, it became located that the concentrations 1 ppm, 5 ppm, and 10 ppm did not kill this species during the treatment period, which is 21 days. When the concentration of 50 ppm is the cause of killing one worm after 14 days of treatment, the reason for the survival of earthworms during the treatment period, especially at low concentrations, may be due to the contact state or the great convergence between the worms and the sources of pollution for a relatively long period of time. The development of its ability to endure was confirmed by Aziz et al. [27]. Earthworms are crucial biomarkers of soil. The bioavailability of mercury depends on speciation, which in turn determines the toxicity, transport, and time remaining for the metal in the environment [28].

Numerous research studies have recognized the dermal uptake of infected soil via means of earthworms [29, 30]. Li et al. [31] found that an increase of cadmium in the soil solution raises the cadmium content of *Eisenia fetida*. Most likely, Saxe et al. [32] reported that cadmium, copper, and lead in earthworms fully aggregate through dermal exposure and that more than 70% of zinc in earthworms is converted into dermal uptake. Furthermore, more than 96% of the mercury in earthworms was converted to percutaneous intake [33]. In a recent study, it was observed that the pattern of uptake and removal kinetics (i.e., rapid accumulation upon exposure, stability at steady state concentration, and rapid excretion at removal phases) became similar between Cr and Cu in *Eisenia andrei* [34]. In addition, the zinc concentration of *Eisenia andrei* stabilized at ~240–420 μg after very rapid absorption of 1–3 days and rapidly decreased to the original level in the exclusion phase [35].

Earthworms are affected by soil contaminants at the various levels of biological organization from sub-organismal, individual to population levels. Pollutants pass through contact through the skin or are ingested and introduced into the viscera [36].

The number of muscle tissue in cutaneous animals exposed to mercury is less than what is controlled, and the *epidermis* and dermis are thinner and more fragile than what is in control, while the adipose tissue of the mercury-exposed group was thicker than the control group, and also the dermal animals exposed to mercury had a smaller number of dermal fibroblasts than the control group in each area [37].

The ability of some worms to survive and live may be due to their adaptive capacity and restore activity, and this is done through their excretion of some substances that have a role in eliminating pollutants, reducing their effects, or their high ability to metabolize these compounds [12, 38].

The results also showed that the concentration of 100 ppm caused the death of 100% of this species within 72 hours, as proven in Table 1, and it is proved that this concentration is the deadly concentration of 1/2 of the number (Lc50) within 48 hours.

The results of the current study showed that all the salt concentrations of mercury chloride (HgCl2) (1, 5, 10, 50, 100) ppm caused a slight decrease in the weights of the worms (Table 2), but in varying proportions, the above concentrations caused a decrease in the weights of the worms by (1.06, 1.03, 0.94, 0.93, 1.11), respectively, as it appeared that the most effective concentrations were 50 ppm. The reason the worms remain in this concentration for the longest possible period throughout the treatment time is 21 days. From this, it is evident that the effect of the salt concentrations of mercury chloride on earthworms is not related to the toxic effect. This is confirmed by Rieder et al. [39] that the loss in live weight of earthworms is independent of the toxic effect of the compound.

The decrease in the weights of the worms may be due to excessive skin secretion when exposed to mercury salts, and this is observed on the worms' bodies when exposed to concentrations throughout the treatment period or as a result of fluid loss due to the imbalance in the hormone responsible for regulating the balance of water and ions, and mercury compounds have the ability to affect the physiological processes of the epidermal cells of living organisms which can increase the secretion of mucus by their glandular cells, and their cells become more clear and transparent [40].

The results of the current study showed that there is an effect of mercury chloride on earthworms, when exposing the worms to the lethal concentration of 100 ppm caused rapid movement and contractions of the worm that included

Table 1: Number of worm deaths exposed to different concentrations of mercury chloride (HgCl2).

Concentrations	The number of worms	Deaths after 24 hours	Deaths after 48 hours	Deaths after 72 hours	Count the worms surviving after 21 days
1 ppm	36	0	0	0	36
5 ppm	36	0	0	0	36
10 ppm	36	0	0	0	36
50 ppm	36	0	0	0	35*
100 ppm	36	6	18	30	0

*One of the worms was killed after 14 days.

Table 2: The average weights of worms in grams, which are exposed to different concentrations of heavy metal salt.

Concentrations	Average weights of worms before treatment (grams)	Average weights of worms after treatment (grams)
Control*	1.22 ± 0.17 b	1.96 ± 0.12 a
1 ppm	1.11 ± 0.12 bcd	1.06 ± 0.18 bcd
5 ppm	1.08 ± 0.16 bcd	1.03 ± 0.19 bcd
10 ppm	1.04 ± 0.12 bcd	0.94 ± 015 cd
50 ppm*	1.14 ± 0.10 bc	0.93 ± 0.12 d
100 ppm	1.12 ± 0.12 bcd	1.11 ± 0.15 bcd

*$P \leq 0.05$.

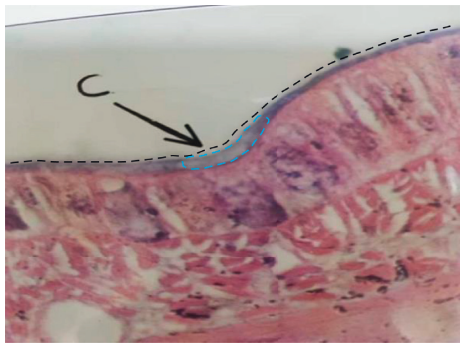

Figure 1: A longitudinal section in the body wall of the control model shows the different tissue layers, and from the top is the cuticle of the connecting tissue. Pigment (H and E) magnification 400.

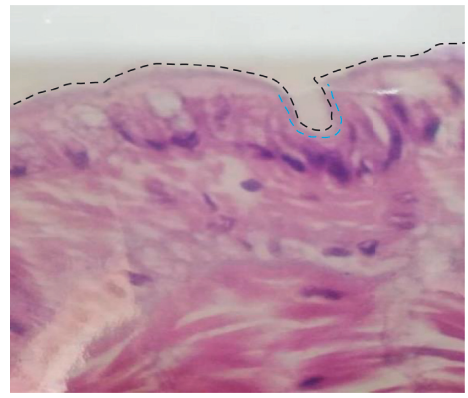

Figure 2: A longitudinal section in the body wall of worms exposed to a concentration of 10 ppm in which the cell boundaries are shrunken and indistinctly defined by the epidermal epithelial tissue (H and E) stain magnification 650.

all areas of the body in addition to the twisting of the worm's body, its blue color, and the appearance of a sticky mucous substance on its body, especially at the clitellum area to become inactive in movement after stiffness and then death. There are many indications that indicate the extent of the effect of heavy metals on living organisms through the occurrence of morphological and functional changes and is then called the response indicator [41].

The results of microscopic examination and study of earthworm skin sections from the area near the clitellum included that the degree of skin tissue cells' susceptibility to different salt concentrations varies according to the concentrations used in the treatment. The histological study also showed that low concentrations of mercury chloride salt had less effect on earthworms' skin tissue compared to higher concentrations.

The impact of mercury salt on the cerebral cells withinside the dermis seems withinside the microscopic exam at a concentration of 10 ppm and that they go through a sizable look change because the outer borders of a number of them turn out to be abnormal, a number of those cells appear like dissolved, the nucleus seems surrounded with the aid of using a small quantity of cytoplasm, and the length and width of the cells are great. Also, the dimensions of the nuclei change, so some of them become irregular in the outer boundaries as well as take a lateral position, and there are some cells that appear to be separate or not based on the basement membrane as well as the lack of pigmentation of the cytoplasm are shown in Figure 1, compared to the control (Figure 2). Also, the concentration of 50 ppm showed that the cells were more affected, as most cells suffer from a clear change in their shape, so they appear irregular in the outer boundaries, and their cytoplasm has a little affinity for pigmentation; this decrease is accompanied by the length of cells, width, length, and width of the nuclei, as in Figures 3 and 4.

The nervous system has a role in the process of exchanging water across the earthworms' body walls [42].

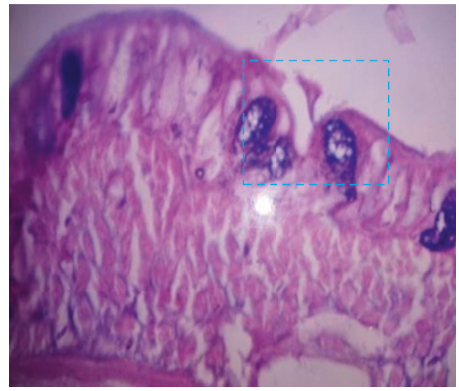

Figure 3: A longitudinal section of the body wall of worms exposed to 50 ppm concentration showing cuticle rupture and cell shrinkage (H and E) magnification 400.

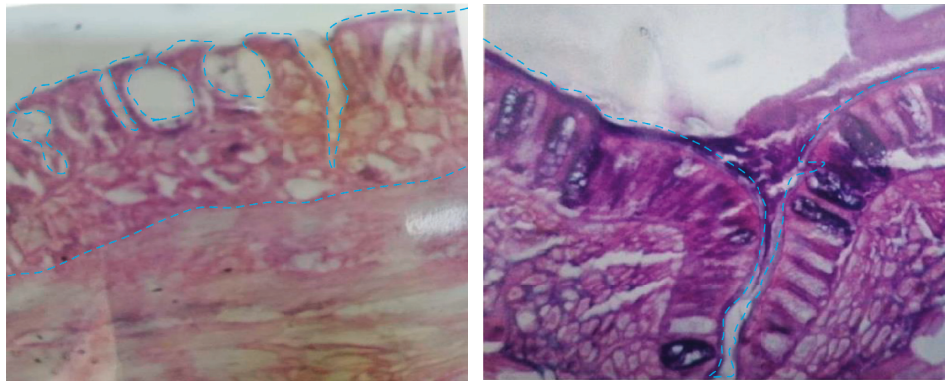

Figure 4: A longitudinal section of the body wall of the group of worms exposed to a concentration of 50 ppm shows cuticle and cuticle rupture, as well as cell shrinkage, laceration, and disappearance of their features. Pigment (H and E) magnification 400.

Chemical compounds and physical factors have the ability to cause phenotypic and structural changes to secretory neurons and their activity.

And this, in turn, is responsible for the physiological processes and the water and ion balance of the worm's body through the cells of the body wall [43]. This may be the reason for the shrinkage of the sizes of epidermal tissue cells due to the loss of cellular fluids.

Earthworms may be dependable signs of soil quality, where the abundance of earthworms and the structure of the community directly reflect the state of pollution in the fields [44]. On the only hand, infected soil influences earthworm feeding and burrowing [45]. This is newly discovered that increasing soil concentrations significantly inhibits earthworm digging activities, with a significantly prolonged avoidance response (from ~10% to >80%) [46]. Wang et al. [47] indicate a decrease in the density of earthworms in soil from 70 to less than 20 worms $m^{2,}$ while Cd content material elevated from 0.81 to over 17.8 mg/kg, and touchy worm species (e.g., *A. homochaetus* and *A. hupeiensis*) steadily faded withinside the infected soils. It is possible that raising the soil lead content from 480 to 5060 mg/kg would significantly reduce the worm density from 135 to 5 m^2 of worms [48].

Accordingly, Asensio et al. [49] evolved an integrative soil evaluation approach via means of comparing the reaction of earthworm's biomarkers (consisting of (Acyl-CoA) oxidase activity, catalase activity, lipofuscin optical density, and the implied epithelial thickness) in the direction of infected soil compared with indigenous worm species; unusual earthworms are normally much less touchy and could exert extra effects on soil properties [50].

Richardson et al. [51] mentioned that earthworms peregrine successfully adapted to soil contaminated with cadmium, mercury, and lead and ultimately affected the cycle of polluted soil. For this reason, the introduction of exotic earthworms is a possible solution to restore soil health [52].

4. Conclusion

The environmental toxicity of soil contaminated with mercury (*Lumbricus terrestris*) was investigated under laboratory conditions for contaminated soils. We concluded that lower worm weights have nothing to do with the lethal concentrations of heavy metal salt (HgCl2). The histological study also showed that low concentrations of mercury chloride salt have less effect on earthworms' skin tissue compared to higher concentrations (it was observed that the contamination of mercury caused a decrease in body mass and earthworms' tissues).

Therefore, the real danger to living organisms lies in the food chain and the accumulation of residues in the tissues of invertebrates, and this will provide a good indicator of the bioavailability of the contaminant and its ecotoxicity.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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