Synergistic Effects of Copper Supplementation and Magnetic Field Exposure on Earthworm Regeneration

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Abstract- Earthworms play a major role in soil functions that help many beneficial ecosystem services. These are important for agroecosystem sustainability. Unfortunately, these services can be damaged by intensive cultural practices like use of pesticides. Earthworm populations are essential decomposers, that contribute to formation of aggregates and nutrient cycling. These involve processes like nitrogen cycles, carbon and phosphorus. These have the ability to influence soil fertility as they contribute to soil structure regulation and organic matter dynamics. They also influence the microbial communities by digestion, stimulation and dispersion in casts. Changes in the earthworm communities are indicators of soil fertility and quality. It is important to understand how earthworm populations affect soil dynamics. Earthworms have an extraordinary capability to regenerate their lost body parts through steps such as: wound healing, dedifferentiation, blastema formation and tissue differentiation. These significant properties make them ideal for studying regenerative biology. It is modulated by both biochemical and biophysical factors. Copper is an essential trace element that serves as a cofactor in several enzyme reactions such as oxidative stress, collagen stabilization, cellular proliferation, angiogenesis and tissue repair. Elevated copper concentrations result in cytotoxic responses implying the need for precise dosage levels. In addition to copper, magnetic exposure is also shown to influence cell behaviour like proliferation, signalling and oxidative balance in invertebrates and mammalian systems. This review examines the effects of copper supplementation and magnetic field exposure on regeneration of earthworms. These provide insights into wound healing and cellular dynamics. However, major research gaps persist, including the lack of standardized experimental protocols, low molecular level analyses and insufficient data on long-term ecological impacts of copper accumulation in soil. A deep understanding of these synergistic mechanisms may help in better understanding of regenerative biology and translational medicine

***Index Terms*—Angiogenesis, Earthworms, Magnetic fields, Regeneration, Toxicity, Wound healing.**

I. INTRODUCTION

Earthworms often referred to as "ecosystem engineers" (Zafar et al., 2025) are the predominant components of the soil biota in terms of maintenance of soil structure, fertility and soil formation. Though they are not high in numbers, due to their large size they have become a major contributor to invertebrate biomass in soils. These activities are important for maintaining soil fertility and play a major

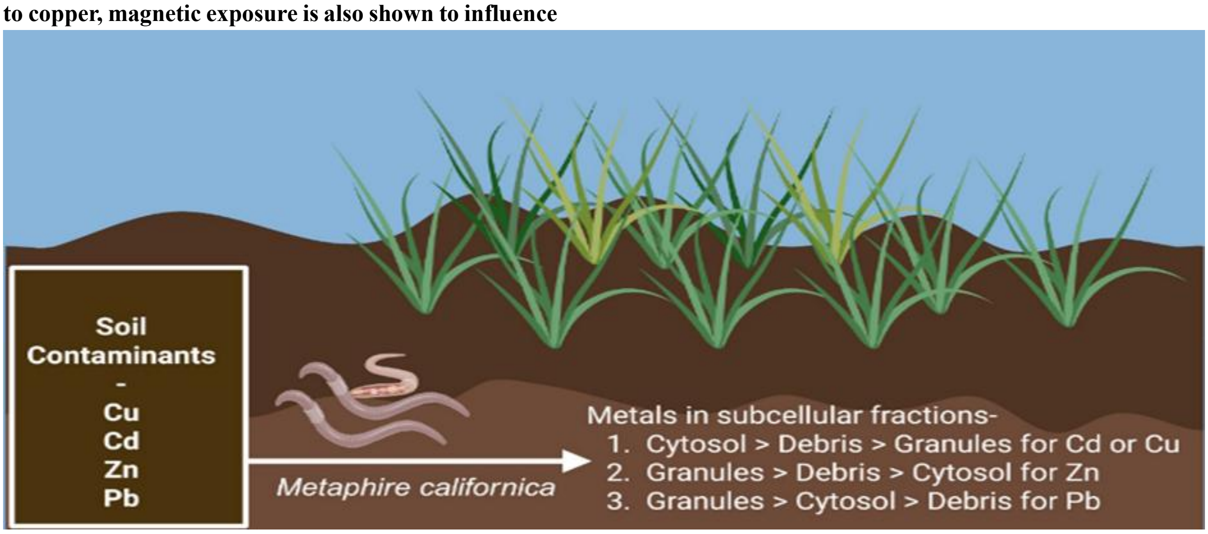

Fig: 1.1: Role of Soil contaminants

role in soil formation (Edwards, 2004). Understanding of factors that affect their survival are necessary. Due to their remarkable availability and regenerative power, annelids are widely used to study regeneration mechanisms as they are easy to handle and culture in the laboratory. Earthworms that hatched from cocoons after a period of 20 days were considered as immature until they have grown a clitellum. These were used for the experiments related to immature regeneration. Adult earthworms, that develop a clitellum after 60 days of hatching, were chosen for the experiments related to adults. Adult earthworms have a well-developed clitellum that consist of seven segments with 26–32 segments before the clitellum followed by 76 segments behind it. It is known that parts of earthworms can survive if they are amputated or cut off (Xiao et al., 2011). Regeneration is a significant ability of an organism to heal and replace its missing body parts. In earthworms, regeneration capability is different among various species. It is essential to study the mechanism behind the regeneration process.

Annelids, nemertean worms, planarians and chordates are few examples with extensive regenerative abilities. Regeneration abilities in earthworms are classified into: (i) clitellum-dependent (ii)clitellum-independent regeneration (Selvan Christyraj et al., 2025). Regeneration abilities are significantly affected by nutrition and temperature. Higher survival rates were observed for earthworms that have the most body segments remaining after amputation. Two lengths of anterior regeneration noted. Regeneration length of body segment 6/7 to further down the body increased gradually (Type L_I). But, the regeneration lengths of earthworm that were amputated behind the 23rd segment, did not grow until the blastema and tail bud formation (Type L_{II}) (Xiao et al., 2011).

II.EARTHWORM REGENERATION: BIOLOGICAL BACKGROUND

Earthworms exhibit bilateral symmetry. They are externally segmented, followed with a corresponding internal segmentation. They lack a skeleton and have a thinly pigmented cuticle. They consist setae on all segments except the first two. They also have an outer layer of circular muscles and an inner layer of longitudinal muscles. Earthworms are hermaphrodite which means they consist both male and female reproductive organs (Edwards & Arancon, 2022). Earthworms (annelidae), consist two fluids, which qualifies as blood. One of these fluids is red which is also free from corpuscles. These corpuscles are specialized cells or cell-like structures found in the body, typically suspended in a fluid like blood or lymph. These are contained in a very extensive series of vessels. The other fluid is colourless and transparent which contains a large number of nucleated cells along with various corpuscles, occupying the entire general or perivisceral cavity. This cavity refers to the region that lies between the digestive tube at the center of the body and the surrounding muscular walls or the external integument that form the body surface, filling the intervening space throughout the organism (Ray Lankester, 1865). Morphologically the body of an earthworm is divided into 120 segments with three main regions: (i) head (ii) clitellum (iii)intestine. The head region comprises of organs that are responsible for the primary functions(Paul et al., 2022). These are called pre-clitellum segments, namely prosto-mium, four pairs of tubular hearts, seminal vesicles, two pairs of testicles, and a pair of ovaries. The girdle like structure beside the pre-clitellum segments is known as clitellum. It plays a major role in reproduction as it contains various glands that help in reproduction. These glands also secrete albumin and mucus. After the clitellum, a pair of prostate glands are present which acts as an auxillary reproductive gland. Anus is located at the dorsal surface. Clitellum is a secretive structure that appears swollen. It is also responsible for the production of mucus, egg capsule protein and albumin. Secretion of albumin is more in adult cells than compared cells. The fertilized eggs are kept in sticky sac near the head. The clitellum is enlarged even more when the worm reaches sexual maturity signifying its adult stage. It can be classified based on its size, color and shape. As they attain sexual maturity their clitellum changes from pink to orange. Earthworm species can be identified using the clitellum segment arrangement. Eisenia foetida has three distinct regions on the clitellum. They are classified based upon the presence of glandular organelles. Namely, (i) Region 1, (ii)Region 2 (iii) Region 3. Earthworms serve as an ideal model for analyzing cellular humoral immune defense responses mediated by leukocytes. It involves wound healing, dedifferentiation, blastema formation and cellular differentiation. Among various environmental and physiological factors, trace elements play a crucial role in regeneration (Selvan Christyraj et al., 2025).

III.ROLE OF COPPER IN BIOLOGICAL SYSTEMS

Accumulation of Copper can take place in agricultural topsoil through the use of Copper based fungicides that might eventually lead to the harm of earthworms (Pelosi et al., 2024). Copper (Cu) is a trace element present in earthworms. It becomes harmful if present in high concentrations. Earthworms are extremely vulnerable to soil contaminants especially to heavy metals such as zinc (Zn) and copper (Cu) that accumulate in soils as a result of industrial and agricultural activities. These metals have a negative effect on earthworm physiology, reproduction and their overall survival. As a result, their essential roles in soil are being affected. Exposure to these heavy metals cause bioaccumulation, oxidative stress, enzyme inhibition and physiological damage. These pose a serious threat to soil health and biodiversity (Zafar et al., 2025). Due to their highly permeable skin and continuous contact with soil as they ingest it during feeding, earthworms are particularly vulnerable to pollutants present in their environment. Heavy metals have the tendency to bioaccumulate within their tissues. This causes significant physiological disturbances and serious health effects. Heavy metals have a serious effect on earthworms at all levels of organizations. They hinder normal enzyme functions, induce genetic damage, lower survival rates, restrict growth and cocoon formation. They are also capable of modifying behavioural patterns, that lead to decrease in the overall diversity and biomass of earthworm populations. Such kind of harmful impacts of heavy metals on earthworms can lead to significant disturbances in population stability. These cause severe ecological repercussions that affect the entire terrestrial ecosystem (Yadav et al., 2023). At recommended dosage levels, copper acts as a cofactor for key enzymes such as superoxide dismutase, lysyl oxidase, and cytochrome c oxidase, supporting processes such as antioxidant defense, collagen

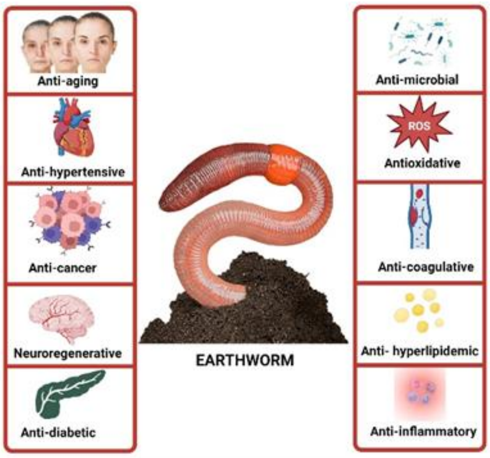

Fig : 3.1 Applications of earthworms

cross-linking, energy production, and cell growth. These roles are important for wound healing and regeneration. Copper helps in new tissue formation, blood vessel growth and restores damaged segments. However, when copper levels rise too high, they become toxic. It stimulates the production of reactive oxygen species (ROS), which causes oxidative stress. This will eventually damage proteins, lipids, and DNA which leads to decrease in regeneration rate. These help to study soil health and act as indicators of pollution as they also accumulate copper from soil. Though after several researches the safe dose of copper in earthworms still remains unknown. The understanding of this balance is vital for earthworm biology offering insights into soil ecology and environmental conditions.

IV.MAGNETIC FIELD INFLUENCE ON BIOLOGICAL TISSUES

The application of magnetic fields for therapeutic effects has seen a rise in recent decades. This has very low side effects making them used widely for physical therapy. Both permanent magnets and alternating magnetic fields are frequently used. Electromagnetic fields is a major form of energy on Earth. An electromagnetic wave is a form of energy transmitted through material system in form of electric and magnetic fields. The vector force of electric and magnetic field is perpendicular to each other and to direction of propagation of wave. The electric field is produced by stationary electric charge. Magnetic field is produced by flowing current. Thermal effects are not produced by low frequencies. These magnetic treatments are widely used due to their effects on biological activity. Growing research indicates that exposure to radiofrequency electromagnetic fields (RF-EMFs) is capable of producing a wide range of biological responses (Tkalec et al., 2013). The ratio of diamagnetics to para and ferromagnetics is equally balanced. These can influence energy state for therapy. Lorentz forces are produced by alternating magnetic fields that make the ions to oscillate around their position. This ultimately results in increased transport in cell membranes (Głąb et al., 2016). Several studies have proven that human and animal cells can be influenced by magnetic fields. They have been associated with uncoupling of free radicals in the cell membranes (Dhawi, 2014). These free radicals get concentrated in the cell membrane and cause oxidative damage to the lipids called as lipid peroxidation (Bradford, 1976; Greenebaum & Barnes, 2018; Repacholi & Greenebaum, 1999). These free radicals cause oxidative stress (Kato et al., 1989; Sahebjamei et al., 2007) that leads to enzymatic activity, gene expression and release of calcium. This eventually affects membrane structure and cell growth leading to cell death. Biological molecules are capable of interacting with different spectra of magnetic fields and their interaction is highly dependent on the intensity of these fields (Hore, 2012). This is caused by magnetic properties that arise from the electron's dipole and spin magnetic moment (Chavda et al., 2022). These molecules are called as paramagnetics or diamagnetics. It has been proven that properties like cytoskeleton (microtubules), cell membrane lipids, DNA strands, and some membrane cause the magnetosensitivity of biological systems (Tota et al., 2024). Electrical properties like membrane potential can be used to characterize cellular membranes (Abdul Kadir et al., 2018). Magentic fields induce changes in cellular membranes of cell organelles like mitochondria. The cytoskeleton also helps to maintain cell division, movement, adhesion and for intracellular trafficking (Fletcher & Mullins, 2010). Research by Zablotskii proved that Magnetic field gradient stimulates cell's paramagnetic properties causing cell adhesion and migration (Zablotskii et al., 2013). The cell remodels itself due to magnetic field intensity where the cytoskeleton also reshapes continuously (Tota et al., 2024). The charges in intracellular Ca2+ influence cytoskeleton organization that lead to cell shape changes (Albuquerque et al., 2016). An experiment also demonstrated that exposure to higher-intensity magnetic fields notably elevated oxidative stress markers in earthworms. These markers include malondialdehyde (MDA), while also disrupting normal neurotransmitter-related activities, including alterations in Ca^{2+}/Mg^{2+}-ATPase function and reductions in acetylcholine levels. In contrast, earthworms subjected to weaker magnetic field intensities exhibited substantially improved physiological and functional performance. These lower-intensity fields contributed to enhanced soil or substrate quality, reflected by a pH value that shifted closer to neutral (around 7), increased availability of essential nutrients, greater humus accumulation, and a noticeably higher germination rate of wheat seedlings, collectively indicating better overall cultivability and ecosystem support (Hou et al., 2025).

V. SYNERGISTIC APPROACH: COPPER + MAGNETIC FIELD

Copper is the most abundant trace metal in living systems followed by zinc and iron. This plays a major role in immune system regulation. Elevated levels of copper have been shown to cause potential toxicity through oxidative stress modulation. Major attention is being drawn towards synergistic effect of copper on various therapies including cancer via oxidative stress (Xie et al., 2023). The combined effects of copper and magnetic fields on lower organisms can result in various biological and chemical processes taking place in them. Copper, an essential micronutrient acts as a major cofactor in enzymes like cytochrome c oxidase, lysyl oxidase, superoxidase dismutase. It contributes to cell growth and repair by helping in angiogenesis, wound healing and connective tissue development. It can also cause toxicity at high levels that can generate reactive oxygen species (ROS), disrupts membranes, denatures proteins and causes DNA damage. Whereas only magnetic field can cause cellular modulation that can influence ion transportation especially calcium channels, ROS balance and signalling pathways. It can also help in regeneration as they can enhance it by cell proliferation and differentiation in organisms like planarians and earthworms. It can also sometimes reduce oxidative stress. Though it might have been shown to increase the oxidative stress due to high intensity fields. Now combining these two effects, it results in enhanced regeneration as magnetic fields help cells to utilize copper better by modulating enzyme activity like copper-dependent superoxide dismutase. It also shows altered ROS balance where copper generates ROS while magnetic fields can enhance or mitigate oxidative stress. (Guan et al., 2024; Kinsey et al., 2023; Zadeh-Haghighi & Simon, 2022) Combined these can create a controlled wound healing or regeneration. It improves cellular uptake by changing membrane permeability affecting copper absorption and distribution inside cells. Both copper and magnetic fields can influence stress response genes, so together they may activate signalling pathways. For example, in earthworms copper aids wound repair and magnetic fields have shown to accelerate regeneration. So, combination might speed up growth of lost segments. In planarians, copper + magnetic field activity may alter neoblast (stem cell) activity. In microalgae and bacteria, growth rate and metabolism can be influenced by altering enzymatic redox states (Balas & Durand, 2016)

VI.CASE STUDIES / EXPERIMENTAL EVIDENCE IN EARTHWORMS

During the use of copper-based fungicides copper accumulates in agricultural topsoil that might harms soil organisms such as earthworms. There have been several researches where the effects of copper on earthworms at different concentrations of copper dosages where found. The critical values of copper were determined that can cause toxicity to earthworms using a meta-analysis that accounts to lethal and sub-lethal effects for different species under several exposure conditions. The most sensitive endpoints were during reproduction and growth like success of hatching and hatchling growth (Pelosi et al., 2024). Copper oxychloride is used as a fungicide at a dosage of 3 g/l for rice fields that gives a concentration of 50% copper that gives 1500 ppm of copper. During 30 days of analysis, it was observed that the weight of earthworm gradually increased overtime in control over 0 days. But growth was seen to be decreased in 10, 20 and 30 ppm exposure to copper. The weight decreased by 21.02%, 45.74%, and 44.20% over initial weight on exposure to 10, 20 and 30 ppm copper respectively by 30 days (Patnaik & Meher, 2023). Another study investigated the changes of metabolites, genes, and gut microorganisms in earthworms that were exposed to 0 (control), 67.58 (low), 168.96 (medium), and 337.92 (high) mg/kg of Cu in soil for 60 days. Differentially expressed genes (DEGs) and differential metabolites (DMs) at the low, medium, and high-level Cu exposure groups were identified Several metabolic pathways are disturbed by copper exposure. Copper exposure significantly decreased the diversity of the intestinal bacteria and affected the of intestinal colonizing bacteria. This resulted in high energy expenditure, inhibited nutrient absorption and fatty acid synthesis, and weakened antioxidant and detoxification abilities, inhibiting the growth of the worm (Zhang et al., 2023). The combination of copper and magnetic fields have shown to improve copper uptake by cells in earthworms, improved regeneration abilities and wound healing abilities. (Balas & Durand, 2016) Exposure to a homogeneous electromagnetic field was carried out in a gigahertz transversal electromagnetic (GTEM) cell as previously reported. Eight earthworms per treatment group were exposed for two hours to continuous radiofrequency electromagnetic fields (RFEMFs) at 900 MHz and field levels of 10, 23, 41, and 120 V m-1 corresponding to the power flux densities of 0.3, 1.4, 4.2, and 38.2 W m-2 respectively. At the field level of 23 V m-1, the effect of longer exposure (four hours), field modulation (80 % amplitude modulation, 1 kHz sinusoidal), and the longevity of effects after 24 h of recovery were investigated (Bourdineaud et al., 2017).

VII.APPLICATIONS AND FUTURE DIRECTIONS

Earthworms are often used in vermicomposting, especially in tropical and sun tropical regions. Use of vermiculture can produce organic wastes resulting in decrease in dependency on chemical fertilizers. They also contribute majorly to livestock feed and are used as a source of nutrient. Although it is very important in ecosystem due to its nutritional and soil burrowing properties, they are also capable of accumulating toxic components from soil. It is exposed mainly to the skin and gastrointestinal tract as they are the main routes of absorption. The ground pollutants called biological and non-biological groups are also accumulated in higher organism levels through food chain. Hence this signifies the importance of prediction of safe dosage levels of copper concentration on earthworms to maintain the balance of ecosystem. Also, it has been shown that earthworms obtained from uncontrolled soil might cause toxic effects for animals and eventually lead to public health concerns. Further the presence of bioactive compounds has been reported by several authors. In Philippines, several indigenous committees have used earthworms in traditional medicine. There exist limited studies on presence of potential pharmaceuticals or vermiceuticals. It is shown to have healing effects for labor pains, stomach ache, arthritis, tooth ache, rheumatic problems. It is also used as a protein source for marine organisms called vermimeal. All these applications signify and emphasize the need for higher regeneration rates in earthworms, determination of safe level dosages of copper treatments and molecular-level analyses (; Guerrero, 2009)

VIII.CONCLUSION

This review aims to analyse the effects of copper on earthworm survival and regeneration rate along with magnetic field supplementation. Weak magnetic fields can alter super dioxide dynamics and affect stem-cell proliferation and regeneration in low level organisms like planarians. These can affect copper-induced oxidative stress depending on field strength, timing, and copper dosage. This is based on ROS signalling. Weak fields can change chemical yields of relevant radicals. High copper levels alter anti-oxidant systems like gut microbiomes, metabolism, regeneration impairment. There are very less direct experiments that combine copper and magnetic fields to study regeneration of earthworms. Existing works focus on only either of them. There is a significant lack of data on multiple copper concentrations, several magnetic field concentrations, exposure times, intensities. This study also aims at making relevant studies for soil ecosystems.

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