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Earthworm *Amynthas morrisi* as a remediation candidate for cadmium uptake from polluted water and soil

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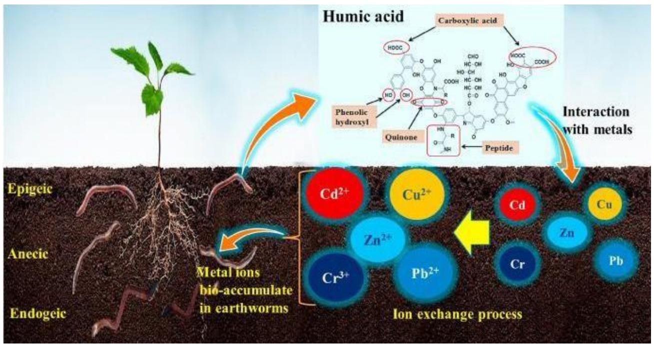
Abstract

Introduction: Cadmium is classified as the fourth toxic element to humans and its presence in soil threatens the food web chain in the ecosystem, especially, human food sources. **Objective:** It was to apply the possibilities of biotic organisms in polluted soil and water. **Methods:** earthworm *Amynthas morrisi* was used in laboratory-controlled cadmium polluted soil/water, to investigate its ability in cadmium uptake via oral ingestion or dermal absorption, after optimizing experiments. In addition, the evaluation of using Laser Induced Breakdown Spectroscopy (LIBS) to monitor the process was conducted along with existing methods, ICP-MS, and XRF. The mortality of *Amynthas morrisi* in various concentrations (0, 5, 10, 15, 20, and 25 μM Cd concentration) at 15 μM Cd concentration (LC50 of *Amynthas morrisi* 7.5 μM Cd), was found able to do a reduction in cadmium concentration from AWW and soil after four days was recoded 7.0 and 3.0 μM Cd, respectively. **Results:** The cadmium accumulated amounts of cadmium (μmol) by *Amynthas morrisi* at the end of the experiments revealed 10.2 and 8.1 of cadmium from AWW and soil, respectively. On the other hand, the investigation of LIBS in the determination of remaining cadmium in the soil or the accumulated concentrations in soil found that LIBS is a direct method in determining cadmium in *Amynthas* morrisi. **In conclusion**, *Amynthas morrisi* can bioremediate cadmium from soil at 7.5 μM Cd, more studies are needed to optimize this process under various conditions. Regarding the suitable method of monitoring the process, XRF and LIBS in the current study were the first to measure the accumulated cadmium in *Amynthas morrisi*.

Keywords: *Amynthas morrisi*. Bioremediation. Cadmium. Soil. Water.

Graphical Abstract



Source: Own authorship.

Introduction

Human being could consume cadmium through contaminated water or food. Its toxicity may cause dangerous health effects. With the development of technology in the field of remedying the cadmium from the environment, especially, water drinking resources and agricultural land, many approaches have been applied. These approaches vary in their basic chemistry, physical, biological, or the combination of some or all of them **[1]**. Biological approach, bioremediation, is the method of applying living organisms to remove cadmium, such as earthworms. It is found that more than 140 studies used earthworms in the remediation of potentially toxic elements **[2]**. Earthworms contribute to cadmium bioremediation through different mechanisms: **a)** availability of free ions of cadmium in the cadmium-contaminated soil to cofactor of soil microflora to remediate cadmium, **b)** ingest cadmium-contaminated soil and bioaccumulate cadmium in their body in certain organs, **c)** through the ingestion of cadmium-contaminated soil, cadmium could be bound with specific molecules (biotransformation), and this complexation of cadmium is the form in which compound form with less bioavailable and toxic, and **d)** earthworms can provide specific environment in soil or water for bacterial flora to enable them do the bioremediation by their other different mechanisms **[1,3]**.

To apply earthworms to remediate cadmium, many aspects must be taken, such as soil composition, their species or ability to do remediation, pH, and organic matter available. Earthworm cadmium bioremediation is low-cost, environmentally friendly, sustainable, and improves soil quality. However, this application of earthworm cadmium bioremediation works with the environment with concentration with their ability to live within and when they exist mainly to do the process in time takes a while but not as rapidly as other processing. In respect to monitoring this remediation process, different methods such as ICP-MS, and recently XRF. Most techniques of measuring cadmium accumulation in biological candidates have shown the challenge in measuring. They involve the preparation of samples before the measuring, which adds other steps in the process and this is mainly acidic as not all the cadmium concentrates are available for detection by an instrument **[4]**.

On the other hand, (LIBS) is an analytical technique that is used in determining the element in environment applications such as soil or dust. This technique can qualify and quantity the material in the environment with no sample preparations needed. Therefore, the current study assumed that the application of LIBS to measure Cd in the earthworm's body could be a new approach to bioremediation processes. *Amynthas morrisi* was obtained from soils and river water. The current study evaluated whether *Amynthas morrisi* is capable of remedying remediation for cadmium and soil. For comparative approaches of determining the accumulated cadmium by earthworms, the existing methods IOPs, XRF, and proposed method LIBS were performed. The prospective use of LIBS for measuring elements within the earthworm's body was discussed using LIBS for direct determination in the environmental sciences. To our knowledge, this is the new application of LIBS performed on an earthworm's body, to date Gaudiuso *et al*., 2010; Zhang, 2021) **[5-7]**.

Material and Methods

Sample collection

The soil sample was collected from the crop field in Hilla (Babylon, Iraq). It was air-dried at room temperature (25) for 4 days. The stones and other materials like leaves were removed and then the soils were sieved using a 2-mm mesh. AWW was prepared as mentioned by [4, *Amynthas morrisi* was collected from the aquatic and agricultural areas and classified due to **[7]**.

The preparation of *Amynthas morrisi* in cadmium-contaminated soil

Amynthas morrisi was used as a candidate to do bioremediation as it survives in cádmium-contaminated soil **[8]**. The earthworms were cultivated in uncontaminated soil before use and the mortality of *Amynthas morrisi* in the most various concentrations (0, 5, 10, 15, 20, and 25 μM Cd concentration) was studied by preparing stock solution of Cd $(NO_3)_2 \cdot 4H_2O$) in sterilized ddH_2O.

Accumulation experiments of cadmium from water and soil by *Amynthas morrisi*

Before conducting experiments, *Amynthas morrisi* were rinsed with distilled water and placed on humid filter paper for 24 h to purify the gut. Tests were represented in an environmental chamber (20 1 C) in darkness. Based on the result of LC50 of *Amynthas morrisi* (7.5 μM), the concentration of cadmium used in the cadmium accumulation experiments was 7.5 μM at pH 7. Briefly, *Amynthas morrisi* (weight 300 mg) was exposed shortly for four days in water or soil nominated with 7.5 μM Cd. Every 10 hours, samples from water and soil were collected. At the end of the experiments, the earthworms were removed, rinsed, and stored at -4 °C.

Determination of Cd concentrations in soil/water and earthworms using ICP-MS

Determination of cadmium in soil/water and earthworms was achieved after *aqua regia* digestion (1V nitric acid (>68%) and 3V hydrochloric acid (<37%), **[1,9,10]**. ICP-MS was certified by assessing linearity, the limit of detection (LOD), limit of quantification (LOQ), precision (relative standard deviation, RSD), and accuracy. Yttrium was used as an external normal during the measurement. To check digestion and analysis accuracy certificate reference material (CRM) market was used as previously **[10]**.

Determination of Cd concentrations in soil and earthworms using XRF and LIBS Spectrometry

A XRF spectrometry (NITON XL31EXRF) was used to determine cadmium in solid phases such as soil and earthworms according to **[9]** LIBS Spectrometry was also used to determine cadmium in solid phases such as soil and earthworms.

Statistical analysis

One-way or two-way ANOVA followed by Tukey's post hoc test, were conducted for cadmium concentrations to assess the differences between the cadmium concentrations measured by various XRF, ICP-MS and LIBS Spectrometry techniques. Software Sigma plot was used to illustrate the results.

Results

The mortality of *Amynthas morrisi* against cadmium

The mortality of *Amynthas morrisi* in cadmium was found at 15 µM Cd and the LC50 was 7.5 µM Cd, as shown in Figures 1A and 1B.

Figure 1A

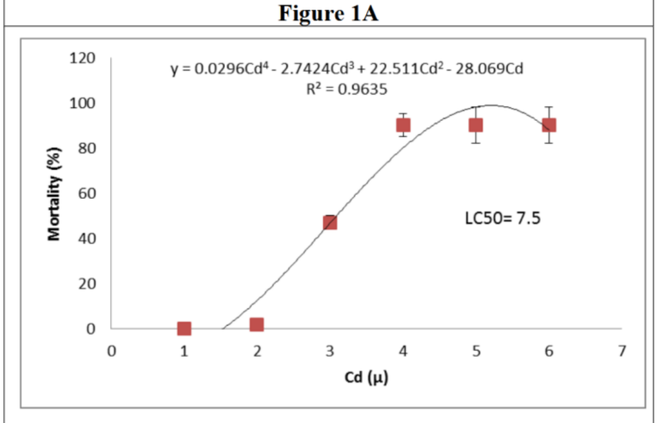


Figure 1A. Mortality of *Amynthas morrisi* under different Cd concentrations. Source: Own authorship.

Figure 1B

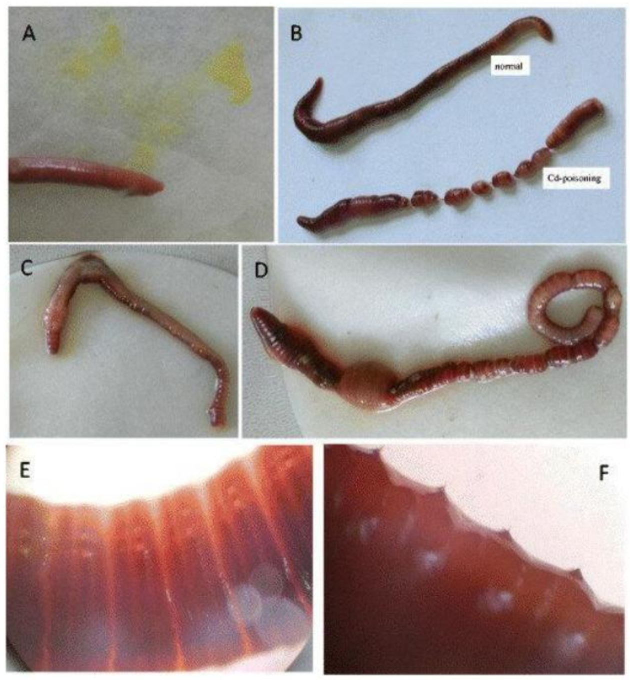
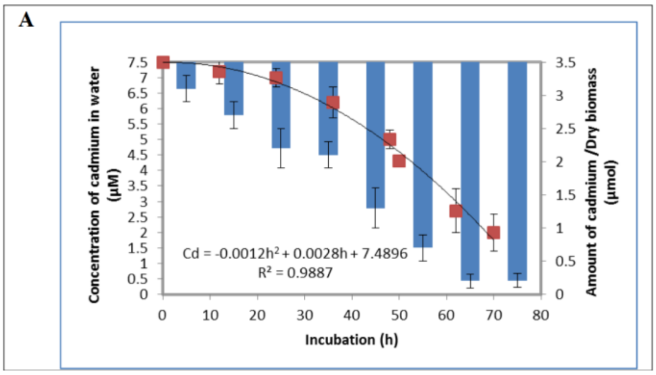


Figure 1B. Mortality increased progressively with Cd concentration, with significant lethality observed at ≥15 µM. The LC50 value (7.5 µM Cd) was selected as the optimal concentration for subsequent accumulation experiments, ensuring organism survival while enabling effective bioremediation assessment. Source: Own authorship.

Accumulation experiments of cadmium from AWW and soil by *Amynthas morrisi*

The reduction of cadmium from AWW and soil showed that *Amynthas morrisi* established cadmium uptake. As presented in figure 2, A and B from an initial 7.5 µM Cd, *Amynthas morrisi* removed 7.0 and 3.0 µM Cd from AWW and soil, respectively. The cadmium accumulated amounts of cadmium (µmol) by *Amynthas morrisi* at the end of the experiments revealed 10.2 and 8.1 of cadmium from AWW and soil, respectively. Accumulation of cadmium from water is more than that from soil free ions of cadmium were more available than in soil media **[11]**.



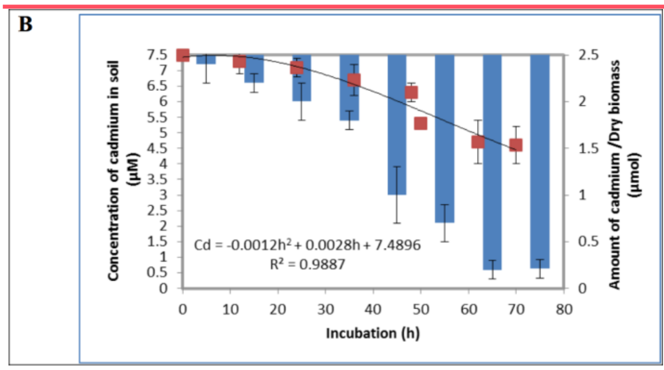


Figure 2. Uptake of cadmium by *Amynthas morrisi* from (A) AAW and (B) soil and nominated with 7.7 µM Cd. The cadmium accumulated amounts of cadmium by *Amynthas morrisi* (■) and residual cadmium in the AAW/ soil (column) were resolute through the incubation. The error bars represent the standard error of the mean (*n* = 3). Source: Own authorship.

Determination of Cd concentrations in soil and earthworms using XRF and LIBS Spectrometry

As shown in Figure 3A and 3B, the comparison between two devices XRF and LIBS Spectrometry presented in addition to the ICP-MS devices. Low concentrations recorded by ICP-MS devices as mentioned before, the acid digestion of the sample prior to the measurement was not enough to release all the elements in the acid phase, and by this, fewer elements were available for detecting.

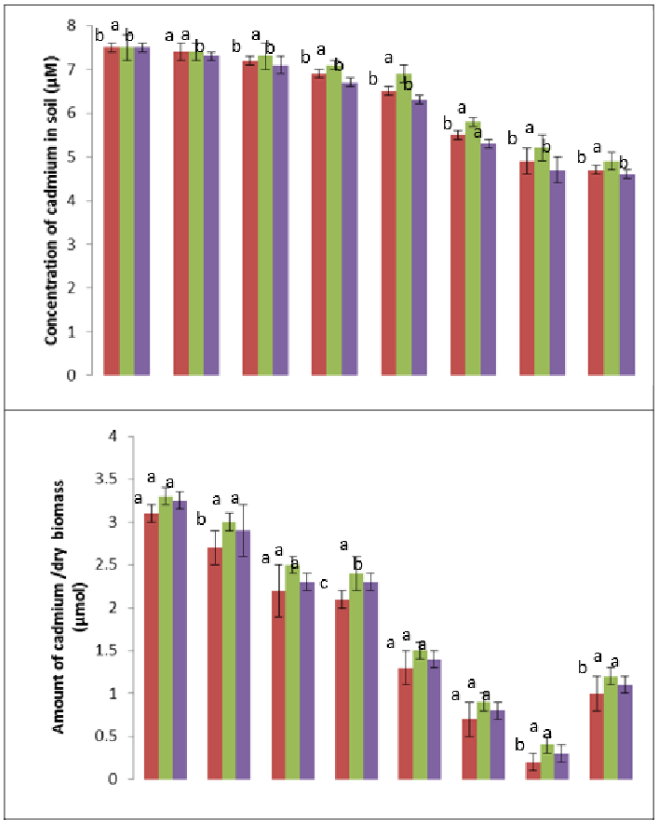


Figure 3. Comparative analysis by ICP-MS (red column), XRF (green column) and LIBS (blue column) of (A) cadmium residual in soil and the amount of cadmium accumulated by (B) *Amynthas morrisi*. relevant to Figure 2. Source: Own authorship.

In contrast, both XRF and LIBS Spectrometry showed significant concentrations compared to ICP-MS devices, as these measurements detect the elements XRF Spectrometry reporting a significant concentration than LIBS.

Discussion

The mortality of *Amynthas morrisi* in cadmium was found similar to the response of earthworms with cadmium recorded by **[4,12-16]**. The reduction of cadmium from AWW and soil showed that *Amynthas morrisi* established cadmium uptake. Accumulation of cadmium from water is more than that from soil free ions of cadmium were more available than in soil media **[11]**. Higher uptake from water reflects greater availability of free Cd^{2+} ions compared with soil, where cadmium is partially immobilized by mineral and organic fractions (Figure 4). Cadmium exists as free Cd^{2+} ions in AWW and as partially immobilized species in soil bound to clay minerals, organic matter, and oxides.

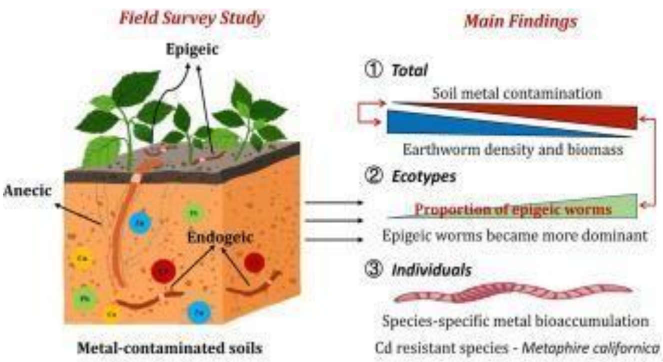


Figure 4. Mechanistic schematic illustrating cadmium uptake and sequestration by *Amynthas morrisi.* Source: Own authorship.

The higher bioavailability of free Cd^{2+} in water explains the enhanced uptake observed experimentally. *Amynthas morrisi* absorbs cadmium through two dominant routes: dermal absorption, Direct diffusion of dissolved Cd^{2+} across the moist epidermis, particularly relevant in aquatic or water-saturated environments, or oral ingestion consumption of cadmium-contaminated soil particles, followed by solubilization in the gut under acidic conditions. internal transport and distribution. Once internalized, cadmium is transported via the coelomic fluid and distributed to metabolically active tissues, particularly: chloragogenous tissue (functional analogue of the vertebrate liver), intestinal epithelium, detoxification and sequestration. Cadmium detoxification occurs through multiple complementary mechanisms: metallothionein binding; Cd^{2+} ions are chelated by cysteine-rich metallothioneins, reducing their reactivity and toxicity. Intracellular compartmentalization, sequestration into vesicles and granules within chloragogenous cells limits interaction with vital cellular processes biotransformation.

Formation of less bioavailable cadmium complexes. Ecological and remediation outcomes: reduction of bioavailable cadmium in soil and water, decreased risk of cadmium transfer to plants and the food chain, improvement of soil biochemical quality and microbial activity and earthworms act as both bioremediators and bioindicators. The comparison between two devices XRF and LIBS Spectrometry in addition to the ICP-MS devices were reported in this study. Low concentrations recorded by ICP-MS devices as mentioned before, the acid digestion of the sample prior to the measurement was not enough to release all the elements in the acid phase, and by this, fewer elements were available for detecting. In contrast, both XRF and LIBS Spectrometry showed significant concentrations compared to ICP-MS devices, as these measurements detect the elements XRF Spectrometry reporting a significant concentration than LIBS. The aim of doing a comparison of both devices in measuring elements in a sample was evaluated recently by **[2,17]**.

Similar findings were evaluated, and XRF shows a more accurate analysis **[4,9]**. LIBS and XRF are both powerful techniques for determining cadmium concentrations in soil. Both techniques do not need any sample preparation and can detect cadmium in low concentrations, in *in-situ* analysis, however, the interference of other presented elements in the soil must be considered in conducting the suitable calibration solutions. Comparing between both techniques, XRF is an *in-situ* analysis of deep soil like for LIBS, and this effect was excluded in the current study.

Conclusion

The earthworm was applied alone in the current study, therefore the investigation of their ability under different complexations model of metals is required to investigate the competition of metals. In addition, the combination with biochar bacteria or organic matter could be approached later for Cd-contaminated soil remediation. The use of LIBS technique in measuring for example elements in earthworms can be applied in the biotechnology filed in terms of remediation or indicator of environmental pollution.

CRediT

Author contributions: Collection of samples, analysis, analysis of data, drafting of the document, and proofreading of the texts was all tasks that were assigned to Zahraa Jawad, Sanaa AL-Muslimawi. The concept was devised by Aminah Murad, Aseel Al-Sabary, Nadia Jebril, and Hiyam Ali, who also oversaw the research and oversaw the reading of the document.

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Ethical Approval

Not applicable.

Informed Consent

Not applicable.

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Data Sharing Statement

Data may be accessible via a responsible request.

Conflict of Interest

The authors declare no competing interests.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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