

EFFECT OF CHROMIUM (Cr⁶⁺) TOXICITY ON EARTHWORM COMMUNITY STRUCTURE ALONG A HEAVY METAL CONTAMINATION TANNERY EFFLUENTS FROM EFFLUENT TREATMENT PLANT IN KANPUR, UTTAR PRADESH

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ABSTRACT

Kanpur is suffering from significant environmental contamination due to the release of toxic metals used in the leather industry, such as Chromium (Cr), copper (Cu), mercury (Hg), and lead (Pb) which are regarded as contaminants. Water and soil pollution caused by industrial effluent emissions is generating serious health problems. Leather industry effluents are among the most toxicological industrial pollutants, containing high levels of Cr⁶⁺, which is extremely hazardous to living organisms. Earthworms are commonly employed as pollution bioindicators in soil environments. To assess the risk of chromium (Cr⁶⁺) pollution, a detailed investigation was conducted along the chromium contamination gradient in different soils on earthworm community structures in Kanpur Uttar Pradesh, India. In accordance with the study, the land closest towards the source of the emission had a very high level of Cr⁶⁺ due to leather processing operations at T1. (520±43.16 ppm and 80±2.32 ppm respectively) and T2 sites (317±37.66 ppm and 59±2.28 ppm respectively). Low occurrence of earthworms occurred in ecosystems with a maximum content of Cr⁶⁺ in T1 and T2 (*E. waltoni* = 113±6.11 and 155±6.11 individuals per m² *M. posthuma* = 23±3.18, 67±6.81 and 168±43.70 individuals per m² respectively). T4 had a maximum mean density of 221±20.34 individuals per m². During the wet season, earthworm density was much higher in Control Site T4, which had the lowest concentration of chromium and was also rich in flora. The species diversity, richness, and biomass of earthworms had a positive relationship with soil and ambient temperature, moisture, pH, and organic carbon. Shannon-Weiner index (H = 0.26) showed the highest density at site T1 and the lowest (H = 0.55) at T1. Site T1 had the highest species richness (I = 0.14) and T2 had the lowest (D = 0.36). Earthworm diversity, richness, and biomass were all closely attributed to the range from the leather processing effluent zone and increased with increasing distance from the tannery effluent area. Cr⁶⁺ levels adjacent to the emission source were substantially high in two of the four land use types studied to be hazardous to earthworms and their habitats. These surveys are important for environmental protection because study sites are irrigated by tannery-treated water and therefore earthworms could be utilized as indicators of polluted sites.

Keywords: *Chromium, Earthworms, Land uses, Contaminants, Tannery effluents*

INTRODUCTION

In the leather tanning business, chromium in hexavalent form is a hazardous toxin in tannery waste and is one of the key elements that should be accounted for in order to maintain environmental and public health. Because of its stabilizing capabilities, chrome tanning currently accounts for 90% of global leather manufacture, allowing leather to resist microbial destruction as well as temperature change, perspiration exposure, and other environmental stressors. Only 20% of the material may be turned into leather, while the remaining is discarded, largely into wastewater (Sahu *et al.*, 2008). As a result, chromium monitoring is required to assure the safety of both the leather itself and the leather tanning effluent in order to safeguard people, ecosystems, water supplies, and agricultural land from harmful levels of chromium exposure while also following local standards.

Long-term discharge of chromium-containing effluent from tannery wastes has contaminated terrestrial and aquatic ecosystems in different regions of the country (Loureiro and Lotade, 2005). Heavy metal contamination in and around soils has been discovered in Uttar Pradesh as a result of the industrialisation of several metropolitan and congested towns with large industrial waste output. However, this problem is exacerbated in Kanpur due to a massive tannery complex with over 450 tannery enterprises. Toxic metals like Cr, As, Fe, and Hg, are discharged into water by the leather industry. The discharge of these industrial effluents is regarded as one of the most contaminated industrial wastes and contains significant levels of metals, generating serious environmental problems that endanger human health, other creatures, and soil ecosystems (Reinecke *et al.*, 1999). As a result, actions are required to reduce the impact of chromium entering the human food chain. The combined assessment of heavy metal impact on earthworm community composition along a pollution gradient indicate that the overall earthworm abundance is inversely proportionate to heavy metal concentrations in soils. Certain earthworm species are particularly vulnerable to the heavy metal contamination of soil and are missing in areas with more resistant species. Differences in abundance and species diversity across a gradient of heavy metal concentration from the source of origin suggest that increased heavy metal content in soil has a major impact on earthworm life activity. Thus, one of the primary factors for determining the soil's favourable physicochemical properties is the earthworm population abundance as well as species composition (Seribekkyzy *et al.*, 2022). Earthworms are widely used as pollution bioindicators of soil ecosystems. Earthworm species that live in metal-contaminated soils have species-specific behaviours that influence how they react to metal pollution (Reinecke *et al.*, 1999). A research investigation was carried out to look into the status of hexavalent chromium Cr⁶⁺ in tannery effluent and contaminated soils of four land use types receiving these treated tannery effluents, as well as changing environmental parameters. The study's goal was to assess the effect of Cr⁶⁺ pollution on the population dynamics and community structure of earthworm species under different land use types. Consequently, the most relevant earthworm species that may be employed as bioindicators for chromium-polluted soils could be identified.

MATERIALS AND METHODS

Study site:

The study was conducted at four sites receiving tannery effluents from a common effluent treatment plant. The sampling sites are situated on the left and right banks of the River Ganges as well as its tributary, the Pandu River (Gowd *et al.*, 2010). Kanpur is located between 80° 21' East longitude and 26° 28' North latitude.

The research site soils receiving the tannery effluents along the gradient from high to low concentration were used to identify the locations (Fig.1). For the past two decades, treated and untreated waste water has been used to irrigate 1800 acres of land (Gupta *et al.*, 2010).



Shekhpur (T1)



Peundi (T2)



Maekupurwa (T3)



Shawaldas Ghat (T4)

Figure 1: Study Sites

- Shekhpur - 0.5 km from the release of tannery effluent (here after referred to as T1).*
- Peundi - 1.5 km from the source of tannery effluent (here after referred to as T2).*
- Maekupurwa- 4 km from the source of tannery effluent (here after referred to as T3).*
- Sawaldas Ghat-10 km from the effluent source and was used as control (here after referred to as T4).*

Earthworm sampling:

Earthworms were collected using the standard technique (Anderson and Ingram, 1993). Detailed description of study sites is as shown (Table 1). Earthworms were collected on a monthly basis using random sampling procedures (five replicates) in a plot of 40 x 50 m² for a period of 12 months (March 2020 - February 2021). Earthworms were extracted using the hand-separating method after excavating a pit of 25 *25*35 cm in soil in four layers of 0–10 cm, 11–21cm, and 22–32 cm. Earthworms were retrieved from each layer, washed, and then narcotized in 70% alcohol for 24 hours. They were weighed and then kept in formalin (4%) for further analysis (Anderson and Ingram, 1993).

Soil Sampling:

Sampling of the soil was done at three distinct depths. Between 0 to 10cm³, then 11 to 21cm³, a third one between 22 to 35 cm³ at regular monthly intervals (March 2020-February 2021) from all sampling sites using a pit size of 25*25*35cm³ (five replicates). The soil samples were airdried, crushed and sieved (2.2mm). These samples were then preserved for further analysis (Okalebo *et al.*, 1994). At 0-10 and 10-20 cm depths, soil temperature and moisture were monitored. Soil moisture was measured by oven-drying soil from two depths at 105 °C and expressed as a percentage of its dry weight. A 1:5 soil-to-water ratio was used to calculate soil pH. The Walkley black method was used to quantify organic carbon (Nelson and Sommers, 1996), and the molybdenum blue method was used to measure available phosphorus in soil (Watanabe and Olsen, 1965). Cr (ppm) was determined by digesting 1 g of the sieved soil samples with a 5:1 combination of concentrated HNO₃ and HClO₄ acid solution until the sample turned transparent for heavy metal analysis (Gupta *et al.*, 2010). This solution was then filtered into a conical flask and diluted to a volume of 50 ml with distilled water, and analysed for Cr using atomic absorption spectrophotometer (Allen, 1989; Madurapperuma and Kumaragamage, 1999). The recovery yield of Cr elemental analysis considering values of CRMs is 0.0005ppm. The certified reference standard used for AAS analysis was K₂Cr₂O₇ (Sigma Aldrich, ≥99% purity). By combining 2.828g of K₂Cr₂O₇ with distilled water and diluting it to 1000 mL, a working standard Cr⁶⁺ solution of 1000 ppm was prepared. This stock solution was then diluted 100 times to create the working standard (1ml=10 ppm Cr⁶⁺). To prepare a range of 0-500 ppm Cr⁶⁺, 1-50 ml of working standards were added and made up to 50 ml with distilled water in volumetric flasks. DTPA solution was used to extract Cr⁶⁺ from the soil samples that had been impacted by tannery effluents. 40 mL of DTPA (0.005 M), CaCl₂ (0.01 M), and triethanolamine (0.1 M) (pH 7.3) were combined with the 4 g of 0.22-mm sieved ground soil samples, and the mixture was mechanically shaken (sample: solution ratio of 10:1 v/w) at 100 rpm for two hours. The blanks were also prepared following the above method. The suspensions were then filtered and stored for further analysis of Cr⁶⁺ by AAS at 357.9nm.

Table 1: The descriptions of study sites along a chromium pollution gradient receiving tannery effluents from a common effluent treatment plant

| Sites | Threshold value of Cr ⁶⁺ | Cr ⁶⁺ (ppm) (mean ±SE) | Pollution level | Land use Type | Vegetation |
|------------------|---|-----------------------------------|---------------------|---|--|
| Shekhpur T1 | 9.07 to 10 ppm Normal range of Cr ⁶⁺ in soil | 520±43.16 | Highly polluted | Land use pattern Located 0.5Km from emission source, with trees and flowering plant. Field is irrigated with treated tannery effluent water round the year accept during rainy season. | <i>Trees:- Ficus religiosa, Azadirachta indica, Pisidium guajava,</i> <i>Shrub:- Brassica oleracea, labiab purpureus, Spinacia oleracea, Cyperus rotundus.</i> |
| Peundi T2 | | 317±37.66 | Moderately polluted | Land use pattern Located 1.5Km from emission source, with trees and flowering plant. Field is irrigated with treated tannery effluent water round the year accept during rainy season. | <i>Trees:- Ficus religiosa, Euclyptus globules, Azadirachta indica, Carica papaya,</i> <i>Shrub:- Tabernaemontana divericat, Tagetes erecta, lagenaria siceraria, Cucurbita pepo, rosa rabiginosa, Hibiscus rosa-sinensis Jasminum sambac</i> |
| Maekupurwa T3 | | 252±34.35 | Polluted | Located 4Km from emission source, This represent the area surround the river when untreated effluent is put in ganga river and due rains become flooded with river water land use involves only forested vegetation. | <i>Vachellia nilotica, Azadirachita indica, Ficus religiosa, Ficus benghalensis, Artemisia vulgaris.</i> |
| Sawaldas Ghat T4 | | 1.0±0.07 | Control | Grassland Located 10 km away from emission source, and was identified as control site which was not impacted by emission source. | <i>Azadirachita indica, Ficus religiosa, Holoptelea integrifolia. Cyperus rotandus,</i> |

STATISTICAL ANALYSIS

Unlike earlier studies, we utilised ANOVA to investigate the effect of chromium (Cr^{6+}) toxicity on the abundance and biomass of earthworm species, as well as differences in soil parameters along a contamination gradient under diverse land uses. ANOVA is a statistical test used to evaluate multiple sample hypotheses and one-way ANOVA was used to compare the statistically significant variations in the effect of chromium toxicity on earthworm community structure/soil physicochemical parameters along a contamination gradient in different land uses. Multiple comparison testing or Newman Keuls multiple range test was performed a test to determine in which two land use types the earthworm abundance or biomass and or various soil parameters differed from each other. The standard error of the mean values was estimated for the replicate data. In all sites, seasonal variation in abundance and biomass of earthworm species was investigated using One Way ANOVA and multiple range test (Newman-Keuls (q)).

To investigate the association between various soil variables and earthworm ecological traits, Pearson correlation coefficients ($P < 0.05$) were calculated as one moved away from the pollutant emission source. Simpson's Diversity Index was used to measure the earthworm diversity that is the number of species as well as the relative abundance of each species present along contamination gradient in different land uses. To analyse the diversity, richness, and evenness of earthworm species the Shannon-Wiener index (H) was utilized. The SPSS 20 software for Windows (Systat Software Inc. SPSS 20) was used for statistical analysis (Zar, 1999).

RESULTS

Soil physico-chemical properties:

The use of treated industrial effluents for irrigation caused the trickling of heavy metals specifically Cr^{6+} in soils of study sites, thereby impacting soil physical, biological, and chemical characteristics. Study showed that soil near emission source was highly contaminated with Cr^{6+} toxicity which declined with an increase in the distance from the emission source.

Table 2: Physico-chemical properties of soil (mg kg⁻¹ dry wt.) across four different study sites receiving tannery effluent (Cr^{6+}) from a common effluent treatment plant. Distances (0.5, 1.5, 4 and 10 km) from emission sources (means \pm SE, N = 4). RSD=relative standard deviation

| Sites | pH | OC% | PO ₄ (kg/ha) | K(kg/ha) | Cr ⁶⁺ (ppm) | RSD% |
|-------|----------------|-----------------|-------------------------|-----------------|------------------------|-------|
| T1 | 8.0 \pm 0.06 | 1.15 \pm 0.16 | 30 \pm 2.72 | 145 \pm 43.87 | 520 \pm 43.16 | 13.74 |
| T2 | 8.0 \pm 0.06 | 1.08 \pm 0.06 | 28 \pm 2.02 | 283 \pm 65.95 | 317 \pm 37.66 | 24.34 |
| T3 | 7.9 \pm 0.07 | 0.72 \pm 0.10 | 28 \pm 2.17 | 115 \pm 12.02 | 252 \pm 34.35 | 19.05 |
| T4 | 7.2 \pm 0.12 | 0.63 \pm 0.08 | 21 \pm 2.17 | 119 \pm 8.86 | 1.0 \pm 0.07 | 14.12 |

In sites T1 and T2, the soil was alkaline (pH 8), compared to T4 (7.2), while close to T3 (7.9), the pH value decreased with increasing distance from the emission source, and was neutral in control site T4 when compared to polluted sites. Sites T1 and T2 had higher organic matter levels (1.15 \pm 0.16 (%) and 1.08 \pm 0.06 (%), respectively) than T3 and T4 (0.72 \pm 0.10 (%)) and 0.63 \pm 0.08 (%), respectively). Soil pH and organic carbon revealed a negative correlation with emission source distance ($r = -0.97$ and -0.89 , respectively), Phosphate level did not differ between sites, although it decreased considerably in control soil ($P < 0.05$) T4, and showed a negative correlation with increasing distance. Potassium content was substantially greater in site T2 but decreased significantly in sites T3 and T4, and it showed a negative correlation with increasing distance ($r = -0.97$). The concentration of chromium differed substantially

between sites ($F_{0.05(1)12,3} = 54.89$). Cr^{6+} concentration was very high ($P < 0.05$) in T1 and T2 sites (520 ± 43.16 ppm and 317 ± 37.66 ppm, respectively), mildly high (250 ± 34.35 ppm) in T3, and comparatively low (1.00 ± 0.07 ppm) in T4 (control). Soil pH, OC%, and phosphate phosphorus demonstrated a positive correlation with Cr^{6+} pollution concentrations in research sites (0.88, 0.88, 0.95, and 0.27, respectively). The level of chromium decreased as one moved away from the tannery effluent channel. Cr concentrations decreased significantly ($F_{0.05(1)12,3} = 54.89$) with the increasing distance from the tannery effluent channel. It showed a negative correlation ($r = -0.95$) to distance. (Table 2, Table 3) to Cr^{6+} .

Table 3: Pearson correlations (r values) between different parameters measured across different sampling sites receiving tannery effluents from a common effluent treatment plant (ns- no significant correlation)

| Correlation | pH | OC% | PO ₄ (kg/ha) | K (kg/ha) | Cr ⁶⁺ (ppm) |
|-------------|-------|-------|-------------------------|-----------|------------------------|
| Distance | -0.97 | -0.89 | -0.97 | -0.49 | -0.95 |
| Cr | 0.88 | 0.88 | 0.95 | 0.27 | ns |

Earthworm Species Composition:

The experimental sites yielded the identification of two species from two families and two genera. Because the species distribution was determined by the concentration of chromium in the study sites, *Eutyphoeus waltoni* of the family Octochaetidae had a wider spread and was present in all sites (T1- 113 ± 6.11 , T2- 155 ± 6.11 , and T4 221 ± 20.34 respectively) except T3. *Metaphire posthuma*, a member of the Megascolecidae family was found at polluted sites (T1), moderately polluted sites (T2), and slightly polluted sites (T3), but was absent in the control T4 site (Table.4).

Table 4. Taxonomic grouping of earthworm across different sampling sites receiving (Cr tannery effluents from a common effluent treatment plant

| Sampling Sites | Taxonomic Groups | Genus/ Species |
|---------------------------|------------------|---------------------------|
| Shekhpur (T1) | Octochaetidae | <i>Eutyphoeus waltoni</i> |
| | Megascolecidae | <i>Metaphire posthuma</i> |
| Peundi (T2) | Octochaetidae | <i>Eutyphoeus waltoni</i> |
| | Megascolecidae | <i>Metaphire posthuma</i> |
| Maekhpurwa (T3) | Megascolecidae | <i>Metaphire posthuma</i> |
| Sawaldas Ghat (T4) | Octochaetidae | <i>Eutyphoeus waltoni</i> |

Community Structure:

In four experimental sites, there was a significant difference in population abundance of *Eutyphoeus waltoni* ($F_{0.05,6,2} = 18.09$, $p < 0.05$). Overall abundance and biomass of *E. waltoni* decreased dramatically as the Cr^{6+} toxicity level increased. The population abundance changed significantly between from a common effluent treatment plant in control site T4 than in polluted T1 ($q_{0.05,6,3} = 8.44$, $p < 0.05$) and moderately polluted sites T2 ($q_{0.05,6,2} = 5.15$, $p < 0.05$), but not statistically different between polluted and moderate polluted sites T2 ($q_{0.05,8,2} = 3.29$, $p < 0.05$). The drop was substantially more pronounced in the case of *E. waltoni* biomass, with significantly lower values in T1 and T2 treatments as compared

to T4 treatment; sub-adults had significantly lower biomass in T1 as compared to T2 and T4 treatment sites (Fig. 1).

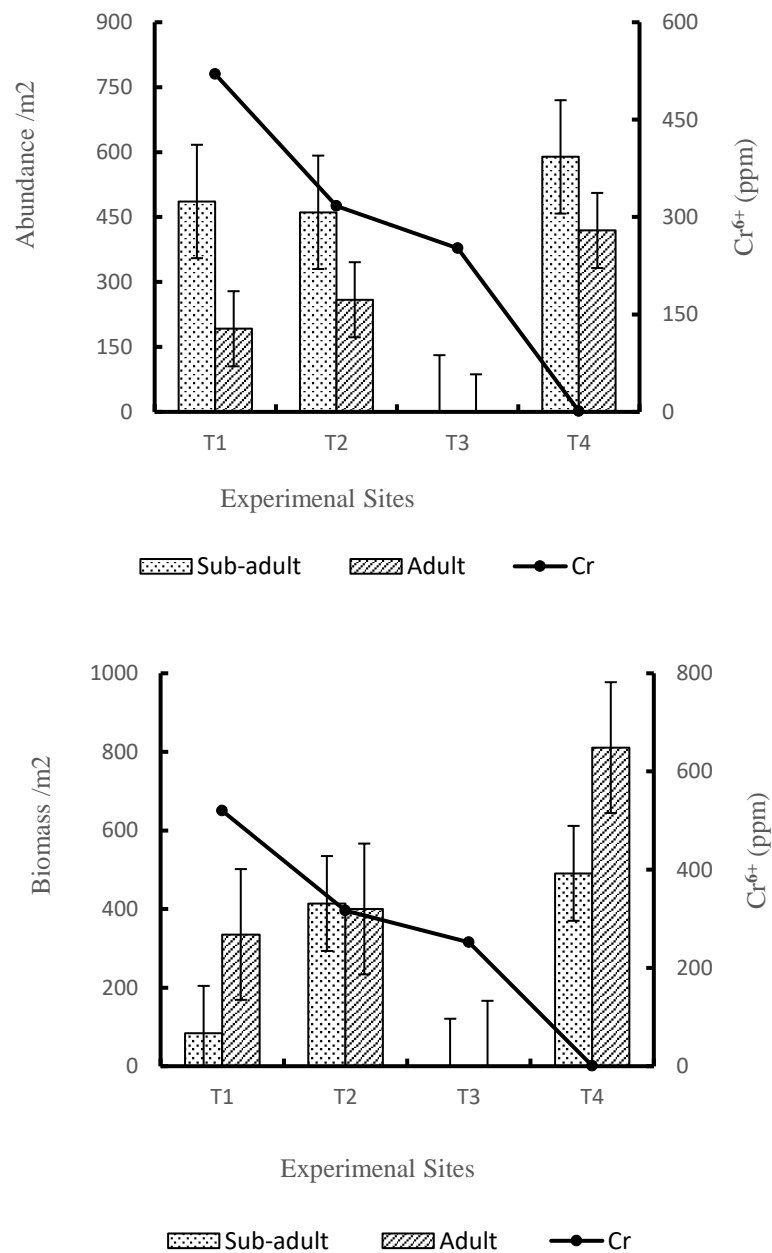


Fig.2: Total abundance (No/m²) and biomass (g/m²) of *E. waltoni* across different sampling sites receiving tannery effluents (Cr⁶⁺ppm) from a common effluent treatment plant.

The total abundance ($F_{0.05, 6, 2} = 15.94, p < 0.05$) and biomass ($F_{0.05, 6, 2} = 19.32, p < 0.05$) of *M. posthuma* varied significantly between highly polluted T1 ($q_{0.05, 6, 2} = 3.09, p < 0.05$), moderately polluted T2 ($q_{0.05, 6, 2} = 4.5, p < 0.05$), and slightly polluted T3 sites ($q_{0.05, 6, 3} = 7.92, p < 0.05$). The population abundance was substantially higher (in minimally contaminated T3 (163 ± 15.39)) and was absent in T4 (Fig.3).

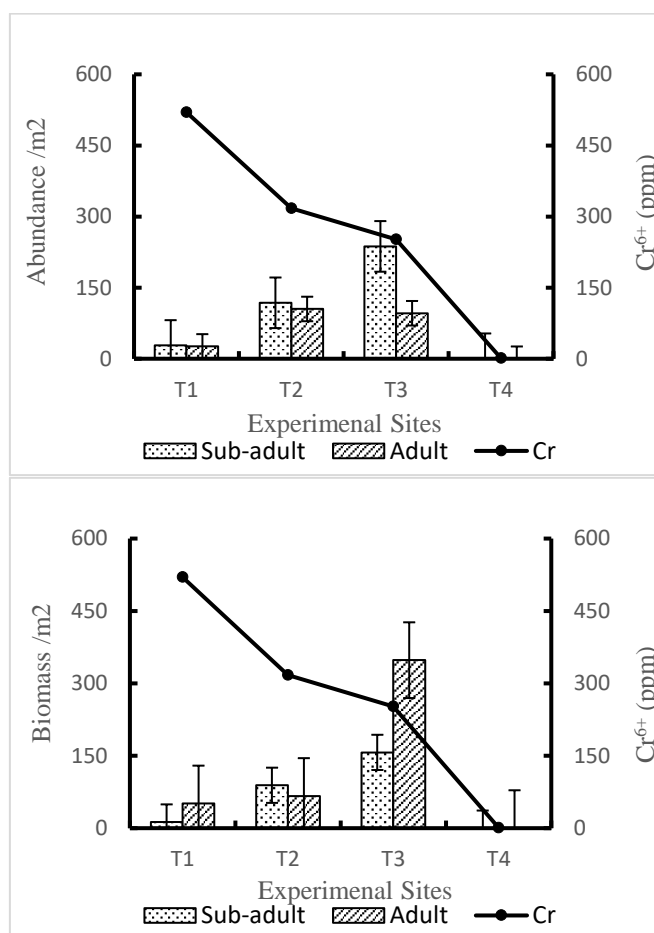


Fig. 3: Total abundance (No/m²) and biomass (g /m²) of *M. posthuma* across different sampling sites receiving tannery effluents (Cr⁶⁺) from a common effluent treatment plant. T1, T2, T3, T4 highlighted in the figure 3 are the treatment sites and uniformly followed throughout the site therefore are not plagiarized

E. waltoni's abundance and biomass were negatively related to Cr⁶⁺ concentrations ($r = -0.93$ and -0.99 , respectively). *M. posthuma*'s total abundance and biomass increased as Cr⁶⁺ concentration levels increased, and it demonstrated a negative association with soil pH, OC%, PO₄, and Cr⁶⁺ levels (-0.95 , -0.98 , -0.74 and -0.88 respectively) (Table 5).

Table 5: Pearson correlations (r values) between soil characteristics and the abundance and biomass of earthworm's species observed at four study sites receiving tannery effluent (Cr⁶⁺ppm) from a common effluent treatment plant.

| Correlation | <i>E. waltoni</i> | | <i>M. posthuma</i> | |
|-------------|-------------------|-------|--------------------|-------|
| | No. | Bio | No. | Bio |
| pH | -0.99 | -0.90 | -0.95 | -0.76 |
| OC% | -0.99 | -0.95 | -0.98 | -0.85 |
| P | -0.98 | -0.97 | -0.74 | -0.67 |
| K | -0.60 | -0.23 | -0.04 | 0 |
| Cr | -0.93 | -0.99 | -0.88 | -0.99 |

Seasonal variations on earthworm species community structure:

The distribution of earthworm species at each location was influenced by the climatic parameters such as the soil temperature and moisture (Fig. 45) as well as such as soil pH, availability of chromium, organic carbon.

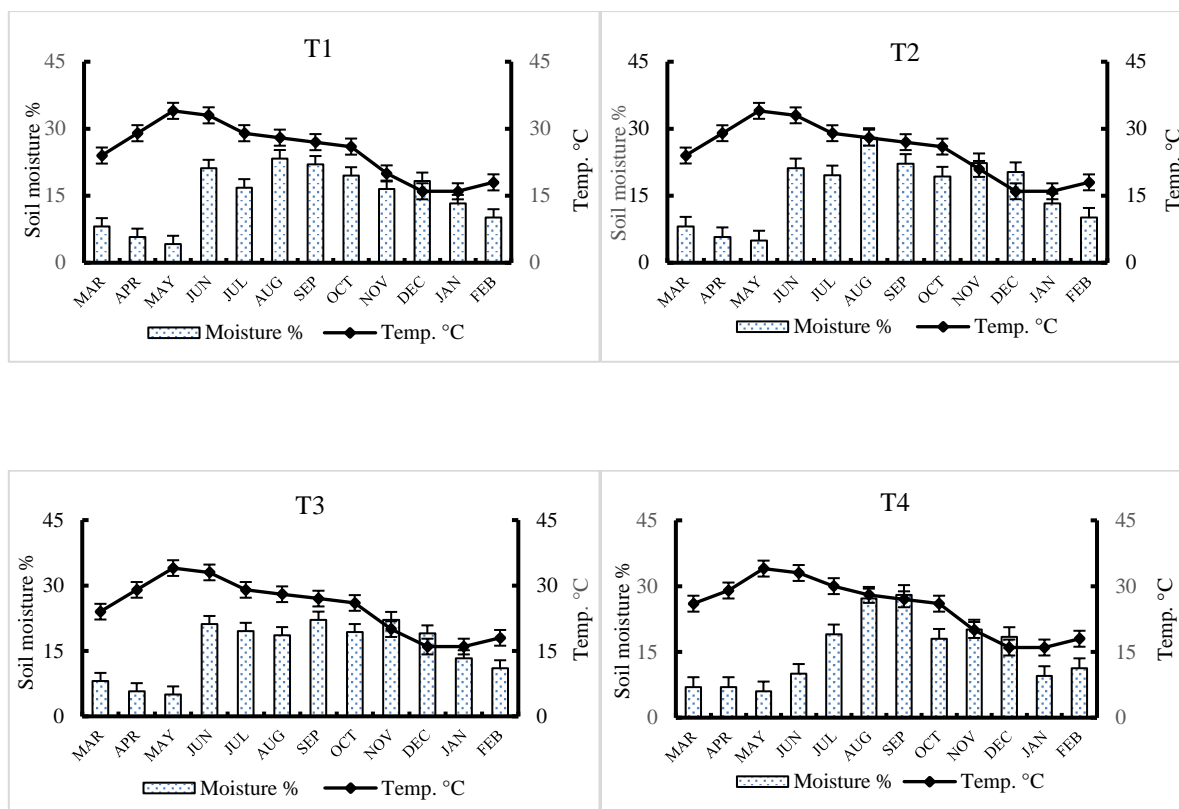


Fig. 4: Monthly fluctuations in soil moisture (%) and temperature (°C) across different sampling sites receiving effluents (Cr⁶⁺ ppm) from a common effluent treatment plant.

E. waltoni was present in T1 sites from July to September, with subadults being much more abundant than adults, but biomass did not differ appreciably. However, once the rainy season began and the Cr⁶⁺ content in the soil decreased, the abundance and biomass of adults increased dramatically, and then reduced as the winter months approached. Subadults outnumbered adults in the moderately contaminated site T2 from July to October, but they had lower biomass values. The abundance and biomass of both adults and juveniles in control T4 were only altered by climatic conditions, with adults having considerably higher abundance and biomass in August and decreasing in September. Nonetheless, subadults peaked in September before declining as winter approached (Fig 5).

M. posthuma sub-adults were substantially more abundant in polluted site T1 during July, although adults showed a peak population expansion during August and were missing throughout subsequent months. Sub-adults were more abundant, but they had extremely low biomass during both months, except in moderately polluted site T2 and mildly polluted site T3. *M. posthuma* was present until October, with peak populations for adults in August and sub-adults in October. However, adults had peak biomass values in September, but sub-adults showed a significant fall in biomass after July. *M. posthuma* showed a similar trend in population expansion in T3 as in T2, but with higher abundance and biomass values (Fig. 6).

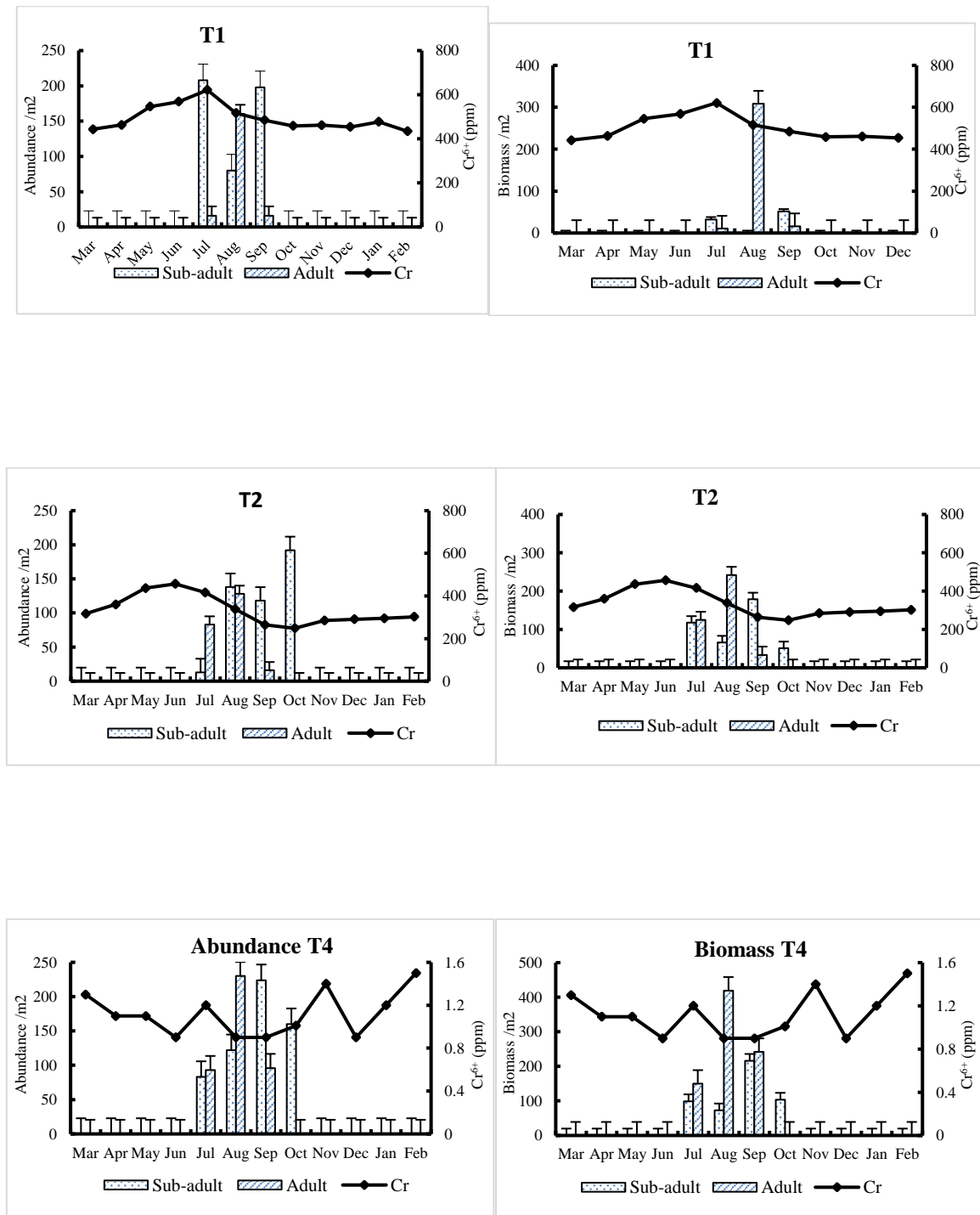


Fig. 5: Seasonal variation in community structure abundance (No/m²) and biomass (g/m²) of *E. waltoni* across different sampling sites (T1, T2, T4) receiving tannery effluents (Cr⁶⁺ppm) from a common effluent plant.

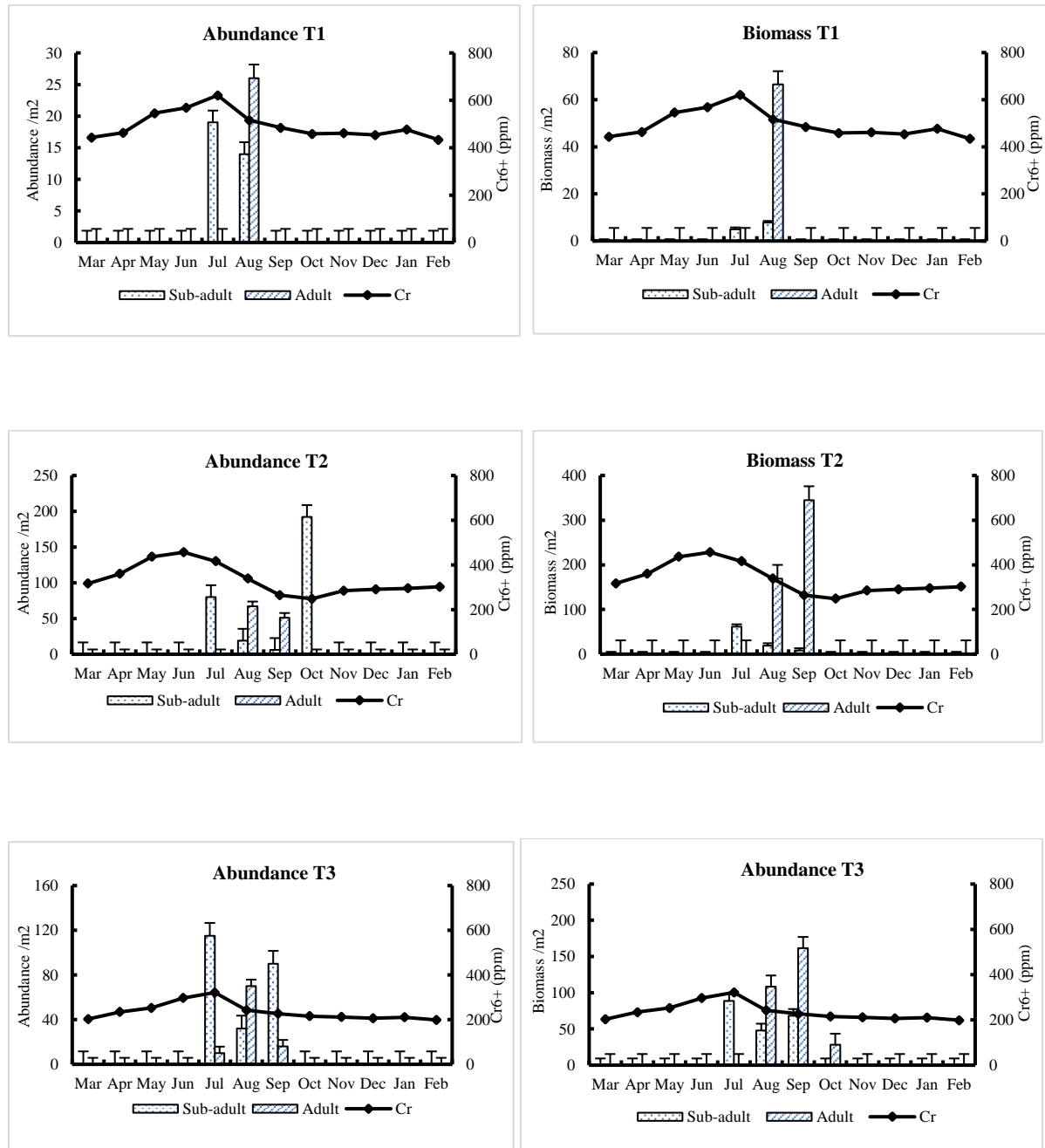


Fig. 6: Seasonal variation in community structure abundance (No/m²) and biomass (g/m²) of *M. posthuma* across different sampling sites (T1, T2, T3) receiving tannery effluents (Cr⁶⁺ ppm) from a common effluent plant.

Total abundance and Biomass:

Total abundance ($F_{0.05,11,3} = 6.77$) and biomass ($F_{0.05,11,3} = 24.73$) of earthworms varied significantly between the four sites and it was significantly higher ($(q_{0.05, 11,4} = 5.59, p < 0.05)$ in T4 site (Fig. 7)

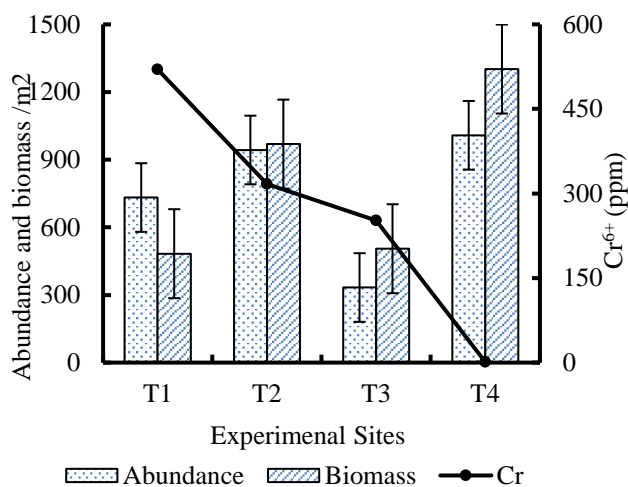


Fig. 7: Total abundance (No/m²) and earthworm biomass (g/m²) at experimental sampling sites receiving tannery effluents (Cr⁶⁺ ppm) from a common effluent treatment plant.

The total abundance and biomass of earthworm species increased with the increase in the distance from the source of abundance ($r = 0.74$ and $r = 0.51$) and a decline in the (Cr⁶⁺ ppm) concentration ($r = -0.84$ and -0.65) (Table. 6)

Table 6: Pearson correlations (r values) between different parameters with total abundance and earthworm biomass measured at experimental sampling sites receiving tannery effluents from a common effluent treatment plant (ns- no significant correlation)

| Correlation | pH | OC% | PO ₄ (kg/ha) | K(kg/ha) | Cr ⁶ (ppm) | Total Abundance | Total Biomass |
|-------------|-------|-------|-------------------------|----------|-----------------------|-----------------|---------------|
| Distance | -0.97 | -0.89 | -0.97 | -0.49 | -0.95 | 0.74 | 0.57 |
| Cr | 0.88 | 0.88 | 0.95 | 0.27 | Ns | -0.84 | -0.65 |

The data analysis revealed that the highest diversity of the species evenness and richness in terms of Shannon-Wiener and Simpson indices were discovered in site T2 ($H = 0.61$ and evenness = 0.58, Richness = 0.42) and the lowest in site T1 ($H = 0.45$ and Evenness = 0.72, Richness = 0.28). More species diversity was indicated by higher values of these indices at site T2. (Table 7).

Table 7: Species diversity index, richness and evenness of earthworm species measured across different sampling sites receiving tannery effluents from a common effluent treatment plant

| Sites | Shannon-Wiener index (H) | | Simpson index (I) | |
|---------|--------------------------|----------|-------------------|--|
| | H | Richness | Evenness | |
| Site T1 | 0.26 | 0.14 | 0.86 | |
| Site T2 | 0.55 | 0.36 | 0.64 | |

DISCUSSION

Contaminants are significantly associated to soils rich in organic matter (Stefanowicz *et al.*, 2020). Soil characteristics, such as Cr⁶⁺, pH, and organic matter, varied amongst the four sites studied, especially when T1 was compared to T4. These components definitely altered soil Cr⁶⁺ pollution patterns and their

impact on earthworms. Based on the proportion of chromium and local soil and vegetation features, earthworm exposure and reactions to Cr⁶⁺ pollution appeared to be location-specific. The negative effects of heavy metals on soil organisms have decreased as a consequence of the substantial organic matter contents at sites T3 and T4. The species diversity, richness, and biomass of earthworms decreased as one moved closer to the tannery effluent canal. These large changes in earthworm communities were caused by the direct and indirect detrimental effects of Cr⁶⁺ (e.g., changes in habitat and substrate quality). Heavy metal contamination has already been observed to produce similar effects in a study done at Harjavalta on soil meso and macrofauna (Lukkari *et al.*, 2004). Earthworms responded to increased Cr⁶⁺ levels at locations T2, T3, and T4, with species numbers and biomass being low at the emission source site T1. The total number of earthworms, on the other hand, increased with increasing distance from the source. The current study found that the distribution, community structure, and activity of earthworms at specific locations were impacted not only by soil Cr⁶⁺ concentration, pH, organic matter, phosphorus, and potassium (Parihar *et al.*, 2019), but also by prevailing climatic and soil variables such as rainfall, ambient temperature, soil temperature, soil moisture, and organic carbon condition (Bhadauria and Saxena, 2018). In comparison to the species *M. posthuma*, *E. waltoni* had higher survival rate in severely contaminated areas (T1 and T2). The absence of *E. waltoni* at T3 could be explained by the regular flooding of this site during rains, which likely inundated their burrows and prevented the species from surviving here (Zorn *et al.*, 2005). *M. posthuma* is an exotic-endogeic species and *E. waltoni* is an endemic-endogeic species, The lower abundance of *M. posthuma* compared to *E. waltoni* could be explained by a competitive exclusion effect because both are endogeic species, but *E. waltoni* is native to the Indo-gangetic plain and hence more adapted to prevailing environmental circumstances, thereby preventing the invasion of exotic *M. posthuma* at T4 sites, but more research is needed (Bhadauria *et al.*, 2021). This was similarly seen in T1, T2, and T3 sites, where *M. posthuma* individuals were less abundant and had lower biomass than *E. waltoni*. Generating a larger number of juveniles by both species is a strategy for species survival under stress conditions caused by Cr⁶⁺ poisoning, this was also observed in the studies done on *O. pattoni* for chromium toxicity (Abbasi and Soni, 1983). However, the reduced biomass of sub-adults under T1, T2, and T3 conditions indicates that both species were exposed to toxicity stress, which altered their feeding behavior and resulted in lower biomass with a greater mortality rate (Liu *et al.*, 2020; Bhadauria *et al.*, 2022). Heavy metals have a deleterious influence on earthworm tissues and organs, such as inhibiting enzyme activity, causing mutations in DNA, disrupting reproductive and survival strategies, altering behavioral patterns, and consequently altering earthworm diversity, community structure, and biomass (Yadav *et al.*, 2023). Another study conducted found that endogeic earthworms are more vulnerable to heavy metal bioaccumulation and that their number decreased significantly in contaminated soils when compared to epigeic earthworms. Investigated. The heavy metal toxicity to earthworms in three regions near to metal industry in Finland revealed that the biomass, survival, and diversity of earthworms declined as distance from metal-contaminated areas increased. This result directly contributes to our findings. Over the past few years, novel ways to assess the existence of toxic metals in soil and water bodies, including apportionment of source analyses employing environmental forensic tools and stable heavy metal isotopes, have been widely adopted. This would aid in identifying the sources of anthropogenic heavy metals that accumulate at any specific site, which might be a critical step in creating successful emission control methods and targeting polluted sites for rehabilitation (Jeong *et al.*, 2021; Wang *et al.*, 2022).

CONCLUSION

The Cr⁶⁺ toxicity generated by the treatment plant that treat tannery effluent has an impact on the characteristics of the earthworm species *E. waltoni* and *M. posthuma*, according to the current study. Earthworm community structure, abundance, biomass, and reproductive activity varied depending on species composition, proximity to the emission source, and physical and chemical soil factors. Climate factors like temperature and precipitation have an impact on how the earthworm community is organized. The endemic, *E. waltoni* and *M. posthuma* were also shown to have a competitive exclusion impact in the study, though further work is needed to confirm this. The study emphasizes that the functional category of an earthworm affects how that species reacts to heavy metal toxicity, with

endogeic species reacting more strongly to heavy metal pollution than epigeic species. The earthworm's reaction to hazardous heavy metals is affected by its proximity to the source of emission. In light of this the study emphasizes the potential of earthworms as bioindicators of heavy metal contamination in any type of land use, with endogeic species better suited for the purpose than epigeic ones.

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