Effect of Biofertilizer on Growth Parameters of *Solanum lycopersicum* grown in Sewage Contaminated Soil of MR 10 Region Indore, Madhya Pradesh and their Forensic Investigation

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ABSTRACT - Soil contamination or soil pollution as part of land degradation is caused by the presence of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals or improper disposal of waste. Metal toxicity or metal poisoning is the toxic effect of certain metals in certain forms and doses on life of plants and human beings. Heavy metals are the major environmental contaminants and possess a severe threat to human, animal and plant health by their long-term persistence in the environment. Pollution from the soil, water and air gets into the food chain by polluting animals and plants that come in contacts with pollutants. The results of present study were important to understand polluted water stress, effect of biological fertilizer (Neem cake powder). Biological fertilizer like Neem cake powder may be used for the suppression of sewage & heavy metal stress. Soil which was irrigated with polluted water has **significantly effect on growth parameters** of tomatoes as compared to plants which are grown in organic field.

KEYWORDS - Sewage, Growth, Organic, Fertilizer, Neem cake powder, Hybrid, Indigenous, Solanum lycopersicum.

INTRODUCTION - Pollution from the soil, water and air gets into the food chain by polluting animals and plants that come in contacts with pollutants. Food pollution can affect us by causing mild to severe illnesses such as hormonal and metabolic problems, various types of cancer. In rare cases when food is highly polluted can causes serious food poisoning. Food pollution can causes anything from small to life-threatening problems. There are different causes of food pollution among them chemical toxins are very harmful and common due to industrialization and urbanization. There may be following situation by which chemical toxins enters into food chain: growing crops in polluted soil or areas with polluted ground water or irrigation with polluted water.

In the present study, tomatoes (*Solanum lycopersicum*) were used and are a member of the *Solanaceae* family. It is a perennial plant and grown as an annual. Tomato plant is a dicot and herbaceous plant. Tomatoes are the major dietary source of the antioxidant lycopene, which has been linked to many health benefits, including reduced risk of heart disease and cancer. Despite botanically being a fruit, it's generally eaten and prepared like a vegetable. They are a great source of vitamin C, potassium, folate, and vitamin K. Tomato is a wonder fruit fortified with health-promoting phytochemicals that are beneficial in preventing important chronic degenerative disorders. Tomato is a plant. The fruit is a familiar vegetable, but the fruit, leaf, and vine are used to make medicine. Tomato is used for preventing cancer of the breast, bladder, cervix, colon and rectum, stomach, lung, ovaries, pancreas, and prostate.

MATERIALS AND METHODS -

1) **Plant:** *Solanum lycopersicum* (Tomato)
2) **Varieties:** Indigenous and Hybrid
3) **Field:** This study has been done in two fields. One was test field- Khatipura near ISBT kumedi under M.R. 10 region of Indore city and another was control- Gram Kaushal, Rangwasa Jaivik Gram Foundation Indore, Madhya Pradesh. Control field was organic and test field was polluted.
4) **Biological fertilizer used:** Neem cake powder- Jaivik Neem Khad.
5) **Seed material:** For the present study, seeds of *Solanum lycopersicum* (Tomato) were collected from Jaivik Setu 66- Bhicholi Mardana, opposite Rajshahi Resort, Bypass Indore Madhya Pradesh.
6) **Crop duration:** In the present study, 70 to 100 days old seedlings were used and the entire test has been done in the fruit of tomatoes.
7) **Sewage Water Analysis:** From Pollution Control Board Indore, M.P. Sewage water analysis involves a range of techniques to assess its quality and composition. These methods are crucial for understanding the pollutants present and determining the effectiveness of treatment processes. Common methods of test like Nitrogen, Phosphorus, Potash, Organic carbon, pH, EC, Calcium, BOD, COD, Magnesium, Sulphur, Fe, Mn, B, Zn and Cu content.
8) **Soil Analysis:** From Polyagro India Pvt. Ltd. District- Baidia, Khargone M.P. Soil analysis methods can be broadly categorized into physical, chemical, and biological tests. These tests are used to determine various properties of the soil, including its composition, texture, and nutrient content, which are crucial for understanding soil health and suitability for various purposes like agriculture or construction.
9) **Heavy Metal Analysis of Tomato Roots and Fruits:** By Atomic Absorption Spectroscopy from Pollution Control Board Indore, M.P. Atomic Absorption Spectroscopy (AAS) is an analytical technique used to determine the concentration of specific heavy metals (**Hg, Pb, Cd, As**) in a sample. It involves measuring the absorption of light by ground-state atoms in the sample. This method is particularly useful for detecting and quantifying heavy metals in various samples like water, soil, and biological materials.

10) **Experimental Design:** Two fields were taken. Out of which one is completely organic considered as control. And another one is polluted field where tomatoes were treated in three ways. Neem cake powder was used in sample I, N.P.K. used in sample II and sample III was only treated with sewage contaminated water.

Table 1: FIELD SET-UP IN SEWAGE CONTAMINATED AND COALMINE'S REGION

S.No.	Set-up	Sample 1	Sample 2	Sample 3
1.	Indigenous Variety of Tomatoes	Sewage contaminated soil	Biofertilizer "Neem cake powder"	Chemical fertilizer "NPK"
2.	Hybrid Variety of Tomatoes	Sewage contaminated soil	Biofertilizer "Neem cake powder"	Chemical fertilizer "NPK"
3.	Tomatoes grown in Coalmine Region	Coalfields soil	Biofertilizer "Neem cake powder"	Chemical fertilizer "NPK"

11) **Parameters Studied:** In the present study, growth parameters like -

a) Germination percentage

By S. Rehman *et al.*, (1998)

⇩

Seeds surface sterilized 0.1% mercuric chloride 5 minutes

⇩

Washed thoroughly sterile water 5 times

⇩

25 thoroughly washed and surfaced sterilized seeds kept in each petriplate 2 layers Whatman filter paper No. 1

⇩

10 ml respective test solution added for fungicide effect determination and for control 10 ml distilled water added

⇩

Petriplate covered kept at room temperature (25 ± 3) in dark

⇩

Grains in which plumage developed into a shoot were considered to have sprouted and the observations on germination were recorded for 7 days.

⇩

The germination percentage was calculated as:

G%= (number of seed germinated/ number of sample seeds) × 100

b) Root and Shoot length - Seedlings measured by using standard centimeter scale.

c) Fresh and Dry weight

Fresh weight - 4 seedlings were selected at random.

⇩

Dry weight - After drying seedlings in a hot air oven 80^{0}C for 24 hours ***(M. Kabir, 2008).*** Both Seedlings recorded using electrical balance.

d) Vigour Index

Calculated by using formula suggested by ***Abdul-Baki and Anderson (1973)*** and expressed in whole number.

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Vigour Index = Germination (%) × (Root length + Shoot length in cm)

OBSERVATIONS AND RESULTS

SEWAGE WATER ANALYSIS –

TABLE 2: SEWAGE WATER ANALYSIS BEFORE GROWING PLANTS

S.NO.	PARAMETERS	NORMAL RANGE	SEWAGE CONTAMINATED WATER	p-value	SIGNIFICANCE (↑ / ↓)
1.	pH	6.5-8.5	9.15	0.00000***	Highly Significant ↑
2.	EC	200-1000	1124.5	0.00000***	Highly Significant ↑
3.	Total hardness	10-1000 (mg/l)	1300	0.00000***	Highly Significant ↑
4.	Calcium	5-200 (mg/l)	475	0.00000***	Highly Significant ↑
5.	Magnesium	50-100 (mg/l)	155	0.00000***	Highly Significant ↑

6.	Chloride	5-1000 (mg/l)	1250	0.00000***	Highly Significant ↑
7.	Sulphate (mg/l)	1-40 (mg/l)	47.870	0.00000***	Highly Significant ↑
8.	B.O.D (mg/l)	1-2000 (mg/l)	2054	0.00000***	Highly Significant ↑
9.	C.O.D (mg/l)	5-1000 (mg/l)	2150	0.00000***	Highly Significant ↑
10.	Cadmium	0 (0.003 mg/L) WHO	0.55	0.00000***	Highly Significant ↑
11.	Lead	0 (0.1 mg/L) CPCB	1.85	0.00000***	Highly Significant ↑
12.	Arsenic	0 (10 µg/L) WHO	25.50	0.00000***	Highly Significant ↑
13.	Mercury	0 (0.001 mg/L) Centre for Science and Environment	350	0.00000***	Highly Significant ↑
14.	Iron	< 0.3 (ppm)	4.1466	0.00000***	Highly Significant ↑
15.	Zinc	5 (mg/L)	25	0.00000***	Highly Significant ↑

SOIL ANALYSIS -

TABLE 3: SOIL ANALYSIS BEFORE GROWING PLANTS

S. No.	Parameter	Organic	Sewage contaminated Test Field (Mean)	After Treated		Significance
				Neem Cake	with NPK	
1.	Nitrogen (kg/ha) (251–400)	325.50	479.33 (p=0.0001)	474.67 (p=0.0002)	481.00 (p=0.0001)	Highly significant ↑
2	Phosphorus (kg/ha) (11–20)	15.50	40.67 (p=0.0023)	38.33 (p=0.0065)	42.00 (p=0.0019)	Significant ↑
3	Potash (kg/ha) (251–400)	325.50	481.33 (p=0.0002)	477.33 (p=0.0001)	480.00 (p=0.0002)	Highly significant ↑
4	Organic carbon (%) (0.5–0.75)	0.62	1.20 (p=0.0380)	1.03 (p=0.0436)	1.20 (p=0.0099)	Significant ↑
5	pH (6.5–8.5)	7.50	9.23 (p=0.0070)	8.73 (p=0.0136)	9.13 (p=0.0078)	Significant ↑

S. No.	Parameter	Organic	Sewage contaminated Test Field (Mean)	After Treated		Significance
				Neem Cake	with NPK	
6	EC (µS/cm) (200–1000)	600.00	1245.67 (p<0.0001)	1235.00 (p<0.0001)	1242.67 (p<0.0001)	Highly significant ↑
7	Calcium (kg/ha) (101–5625)	2863.00	7371.67 (p<0.0001)	7353.00 (p<0.0001)	7361.00 (p<0.0001)	Highly significant ↑
8	Magnesium (kg/ha) (180–1350)	765.00	1396.00 (p<0.0001)	1357.00 (p<0.0001)	1390.00 (p<0.0001)	Highly significant ↑
9	Sulphur (kg/ha) (20–30)	25.00	34.33 (p=0.0441)	31.00 (p=0.1022)	35.33 (p=0.0133)	Sewage/NPK: Significant ↑
10	Zinc (ppm) (0.60)	0.60	0.33 (p=0.0041)	0.31 (p=0.0028)	0.33 (p=0.0020)	Significant ↓
11	Boron (ppm) (0.50)	0.50	0.18 (p=0.0042)	0.15 (p=0.0018)	0.17 (p=0.0028)	Significant ↓
12	Iron (ppm) (4.50)	4.50	4.71 (p=0.0046)	4.68 (p=0.0067)	4.71 (p=0.0125)	Significant ↓
13	Manganese (ppm) (1.00)	1.00	1.32 (p=0.0042)	1.30 (p=0.0015)	1.31 (p=0.0024)	Significant ↓
14	Copper (ppm) (0.20)	0.20	0.22 (p=0.2999)	0.18 (p=0.2495)	0.21 (p=0.3828)	Significant ↓

TABLE 4: SOIL ANALYSIS AFTER GROWING PLANTS

S. No.	Parameter	Organic	Sewage contaminated Test Field (Mean)	After Treated		Significance (vs table 3)
				Neem Cake	with NPK	
1.	Nitrogen (kg/ha)	325.50	440.98 (p=0.0001)	417.71 (p=0.0002)	452.14 (p=0.0001)	Highly significant ↑
2	Phosphorus (kg/ha)	15.50	37.42 (p=0.0023)	33.73 (p=0.0065)	39.48 (p=0.0019)	Significant ↑
3	Potash (kg/ha)	325.50	442.82 (p=0.0002)	420.05 (p=0.0001)	451.20 (p=0.0002)	Highly significant ↑
4	Organic carbon (%)	0.62	1.10 (p=0.0380)	0.91 (p=0.0436)	1.06 (p=0.0099)	Significant ↑
5	pH	7.50	8.50 (p=0.0070)	7.68 (p=0.0136)	8.58 (p=0.0078)	Significant ↑
6	EC (µS/cm)	600.00	1146.62 (p<0.0001)	1084.36 (p<0.0001)	1168.20 (p<0.0001)	Highly significant ↑
7	Calcium (kg/ha)	2863.00	6792.34 (p<0.0001)	6472.76 (p<0.0001)	6918.34 (p<0.0001)	Highly significant ↑
8	Magnesium (kg/ha)	765.00	1283.52 (p<0.0001)	1225.28 (p<0.0001)	1305.86 (p<0.0001)	Highly significant ↑
9	Sulphur (kg/ha)	25.00	31.61 (p=0.0441)	27.28 (p=0.1022)	33.22 (p=0.0133)	Significant ↑
10	Zinc (ppm)	0.60	0.30 (p=0.0041)	0.27 (p=0.0028)	0.31 (p=0.0020)	Significant ↓
11	Boron (ppm)	0.50	0.16 (p=0.0042)	0.13 (p=0.0018)	0.16 (p=0.0028)	Significant ↓
12	Iron (ppm)	4.50	4.33 (p=0.0046)	4.12 (p=0.0067)	4.43 (p=0.0125)	Significant ↓
13	Manganese (ppm)	1.00	1.21 (p=0.0042	1.15 (p=0.0015)	1.23 (p=0.0024)	Significant ↓
14	Copper (ppm)	0.20	0.20 (p=0.2999)	0.16 (p=0.2495)	0.20 (p=0.3828)	Not significant

TABLE 5: SOIL ANALYSIS IN COALFIELDS BEFORE AND AFTER TREATMENT

S. No.	Parameter	STANDARD RANGE	(BEFORE TREATED) COALFIELDS SOIL (Mean ± SD and p-value)	AFTER TREATED WITH		SIGNIFICANCE (↑ / ↓)
				(NEEM CAKE) (Mean ± SD)	(NPK) (Mean ± SD)	
1.	Nitrogen (kg/ha)	251–400	476 ± 3 (9.30×10^{-6})	462 ± 2 (1.00×10^{-5})	470 ± 2 (3.50×10^{-6})	↑ Highly Significant
2	Phosphorus (kg/ha)	11–20	37 ± 2 (0.0015)	33 ± 2 (0.0012)	39 ± 1 (0.0002)	↑ Significant
3	Potash (kg/ha)	251–400	477 ± 2 (5.86×10^{-6})	445 ± 2 (1.07×10^{-5})	472 ± 2 (9.45×10^{-6})	↑ Highly Significant
4	Organic carbon (%)	0.5–0.75	1.4 ± 0.2 (0.0067)	1.2 ± 0.1 (0.0060)	1.3 ± 0.1 (0.0043)	↑ Significant
5	pH	6.5–8.5	9.2 ± 0.2 (0.00025)	8.5 ± 0.2 (0.00029)	9.0 ± 0.1 (4.11×10^{-5})	↑ Significant
6	EC (µS/cm)	200–1000	1244 ± 3 (2.01×10^{-6})	1222 ± 2 (9.66×10^{-7})	1240 ± 2 (8.67×10^{-7})	↑ Highly
7	Calcium (kg/ha)	101–5625	7365 ± 4 (7.58×10^{-8})	7357 ± 3 (2.46×10^{-8})	7353 ± 3 (3.91×10^{-8})	↑ Highly
8	| Magnesium (kg/ha)	180–1350	1395 ± 2 (6.85×10^{-7})	1354 ± 2 (7.87×10^{-7})	1385 ± 2 (1.10×10^{-6})	↑ Highly
9	Sulphur (kg/ha)	20–30	35 ± 2 (0.0035)	30 ± 1 (0.0008)	34 ± 2 (0.0026)	↑ Significant
10	Zinc (ppm)	0.60	0.35 ± 0.03 (0.0045	0.30 ± 0.02 (0.0024)	0.32 ± 0.02 (0.0013)	↓ Significant
11	Boron (ppm)	0.50	0.18 ± 0.02 (0.0069	0.15 ± 0.02 (0.0187)	0.14 ± 0.01 (0.0051)	↓ Significant
12	Iron (ppm)	4.50	4.73 ± 0.02 (6.47×10^{-6})	4.59 ± 0.02 (1.00×10^{-5})	4.67 ± 0.02 (6.11×10^{-6})	↑ Significant
13	Manganese (ppm)	1.00	1.32 ± 0.03 (0.00017	1.25 ± 0.02 (0.00035)	1.30 ± 0.02 (0.00018)	↑ Significant
14	Copper (ppm)	0.20	0.22 ± 0.02 (0.0042)	0.18 ± 0.02 (0.0067)	0.20 ± 0.01 (0.0008)	Not significant

HEAVY METAL ANALYSIS OF SOIL -

TABLE 6: HEAVY METAL ANALYSIS OF SEWAGE CONTAMINATED SOIL BEFORE GROWING PLANTS

S. No.	Parameter	ORGANIC SOIL	SEWAGE CONTAMINATED SOIL (Mean ± SD)	AFTER TREATED WITH FERTILIZERS (Mean ± SD)		SIGNIFICANCE (↑ / ↓)
				Neem Cake Powder	NPK	
1.	Lead (Pb)	0	8.50 ± 0.05 (0.001)	5.50 ± 0.08 (0.005)	6.10 ± 0.06 (0.004)	↑ vs organic; ↓ with Neem/NPK
2	Arsenic (As)	0	0.36 ± 0.02 (0.0011)	0.31 ± 0.02 (0.0008)	0.33 ± 0.02 (0.0013)	↑ vs organic; ↓ with Neem/NPK
3	Cadmium (Cd)	0	0.43 ± 0.03 (0.0017)	0.36 ± 0.02 (0.0017)	0.41 ± 0.02 (0.0003)	↑ vs organic; ↓ with Neem/NPK
4	Mercury (Hg)	0	0.258 ± 0.004 (8.2×10^{-5})	0.251 ± 0.003 (3.3×10^{-5})	0.245 ± 0.003 (5.8×10^{-5})	↑ vs organic; ↓ with Neem/NPK

TABLE 7: HEAVY METAL ANALYSIS OF SEWAGE CONTAMINATED SOIL AFTER GROWING PLANTS

S. No.	Parameter	ORGANIC SOIL	SEWAGE CONTAMINATED SOIL (Mean ± SD)	AFTER TREATED WITH FERTILIZERS (Mean ± SD)		SIGNIFICANCE (↑ / ↓)
				Neem Cake Powder	NPK	
1.	Lead (Pb)	0	5.20 ± 0.05 (1.2×10^{-4})	3.80 ± 0.03 (9.0×10^{-5})	4.10 ± 0.04 (8.5×10^{-5})	↓ Significant
2	Arsenic (As)	0	0.05 ± 0.01 (0.0011)	0.03 ± 0.01 (0.0010)	0.04 ± 0.01 (0.0012)	↓ Significant
3	Cadmium (Cd)	0	0.07 ± 0.01 (0.0009)	0.05 ± 0.01 (0.0008)	0.06 ± 0.01 (0.0009)	↓ Significant
4	Mercury (Hg)	0	0.03 ± 0.01 (0.0008)	0.02 ± 0.01 (0.0007)	0.02 ± 0.01 (0.0007)	↓ Significant

TABLE 8: HEAVY METAL ANALYSIS OF COALFIELDS PLANT'S GROWN ROOTS

S. No.	Parameter	ORGANIC (mg/kg)	COALFIELD ROOTS (MG/KG)	AFTER TREATED WITH		P-VALUE	SIGNIFICANCE (↑ / ↓)
				NEEM TREATED (mg/kg)	NPK TREATED (mg/kg		
1.	Lead (Pb)	0	8.37 ± 0.02	6.56 ± 0.02	8.17 ± 0.02	2.17×10^{-6}	↑ Significant

2	Arsenic (As)	0	3.23 ± 0.03	2.15 ± 0.03	3.26 ± 0.02	7.30×10⁻⁶	↑ Significant
3	Cadmium (Cd)	0	2.11 ± 0.03	1.70 ± 0.02	2.44 ± 0.03	5.21×10⁻³	↑ Significant
4	Mercury (Hg)	0	1.18 ± 0.01	1.16 ± 0.03	1.15 ± 0.02	1.60×10⁻⁴	↑ Significant

TABLE 9: HEAVY METAL ANALYSIS OF SEWAGE CONTAMINATED GROWN FRUITS

S. No.	Parameter	ORGANIC SOIL (mg/kg)	SEWAGE CONTAMINATED FRUITS (MG/KG)	AFTER TREATED WITH FERTILIZERS (mg/kg)		P-VALUE	SIGNIFICANCE (↑ / ↓)
				NEEM TREATED (mg/kg)	NPK TREATED (mg/kg		
1.	Lead (Pb)	0	6.60 ± 0.05	6.07 ± 0.05	6.45 ± 0.02	0.0001	↑ Significant
2	Arsenic (As)	0	2.95 ± 0.02	2.15 ± 0.03	2.88 ± 0.02	0.0021	↑ Significant
3	Cadmium (Cd)	0	1.84 ± 0.03	1.22 ± 0.02	1.55 ± 0.03	|0.0014	↑ Significant
4	Mercury (Hg)	0	1.19 ± 0.02	1.12 ± 0.02	1.18 ± 0.02	0.0010	↑ Significant

TABLE 10: HEAVY METAL ANALYSIS OF COALFIELDS GROWN FRUITS

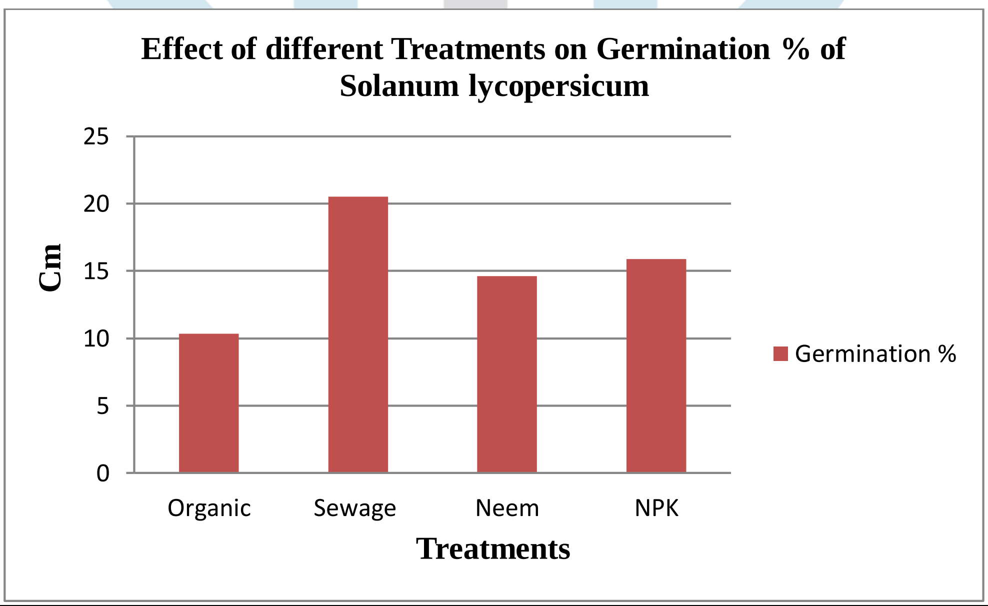
S. No.	Parameter	ORGANIC SOIL (mg/kg)	CONTAMINATED FRUITS (MG/KG)	AFTER TREATED WITH		P-VALUE	SIGNIFICANCE (↑ / ↓)
				NEEM TREATED (mg/kg)	NPK TREATED (mg/kg		
1.	Lead (Pb)	0	6.57 ± 0.02	4.16 ± 0.02	6.42 ± 0.02	5.12×10⁻⁶	↑ Significant
2	Arsenic (As)	0	2.93 ± 0.02	1.12 ± 0.02	2.85 ± 0.02	3.69×10⁻⁵	↑ Significant
3	Cadmium	0	1.82 ± 0.02	1.15 ± 0.01	1.54 ± 0.02	3.29×10⁻⁵	↑ Significant

	(Cd)						
4	**Mercury (Hg)**	0	**1.17 ± 0.03**	**1.21 ± 0.03**	**1.15 ± 0.02**	**1.11×10⁻⁴**	**↑ Significant**

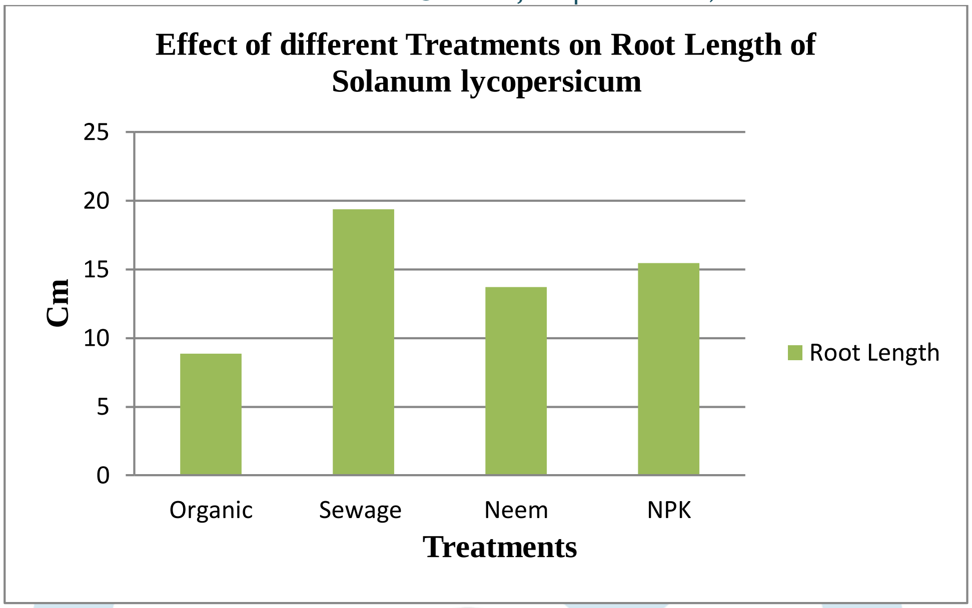
PICTURE 1: SHOWING 70 TO 100 DAYS OLD SEEDLINGS, GERMINATION AND ROOT AND SHOOT LENGTH

TABLE 11: RESULTS AND FINDINGS OF GROWTH PARAMETERS OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZERS TREATED SOIL

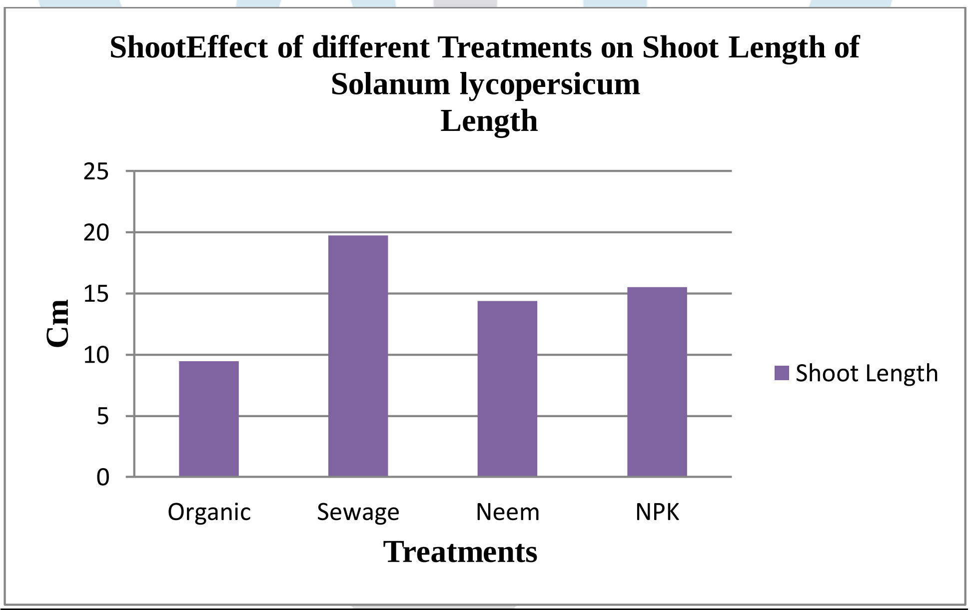
S. No.	Parameters	Organic Soil	Sewage Contaminated Soil	After Treated with		Significance (↑ / ↓)
				Neem Cake	NPK	
1.	Germination %	10.34	20.53 (p=0.0047)	14.63 (p=0.0101)	15.87 (p=0.0003)	↑ Significant
2	Root Length (cm)	8.87	19.37 (p=0.0040)	13.72 (p=0.0143)	15.47 (p=0.0023)	↑ Significant
3	Shoot Length (cm)	9.47	19.74 (p=0.0001)	14.41 (p=0.0100)	15.51 (p=0.0034)	↑ Significant
4	Fresh Weight (g)	8.97	20.56 (p=0.0008)	15.96 (p=0.0013)	16.84 (p=0.0021)	↑ Significant
5	Dry Weight (g)	9.63	19.77 (p=0.0004)	14.37 (p=0.0072)	16.33 (p=0.0032)	↑ Significant
6	Vigour Index	9.41	20.49 (p=0.0081)	14.43 (p=0.0003)	16.42 (p=0.0031)	↑ Significant



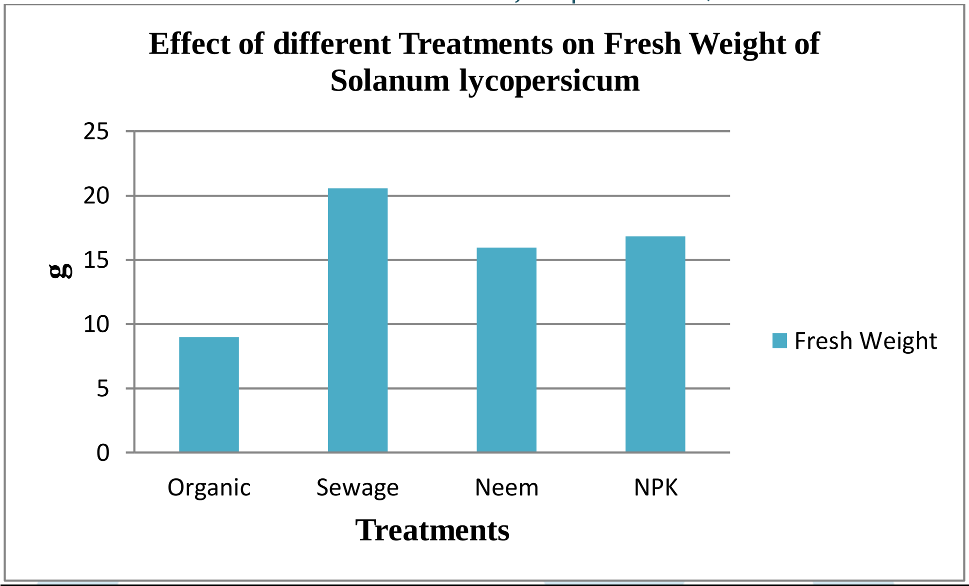
GRAPH 1: SHOWING DIFFERENT TREATMENTS ON GERMINATION % OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZER TREATED SOIL



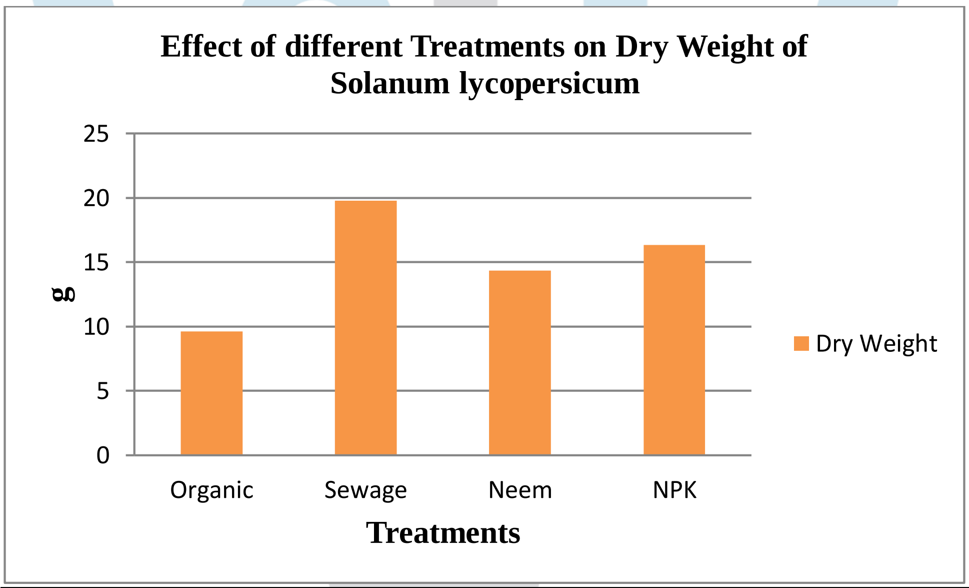
GRAPH 2: SHOWING DIFFERENT TREATMENTS ON ROOT LENGTH OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZER TREATED SOIL



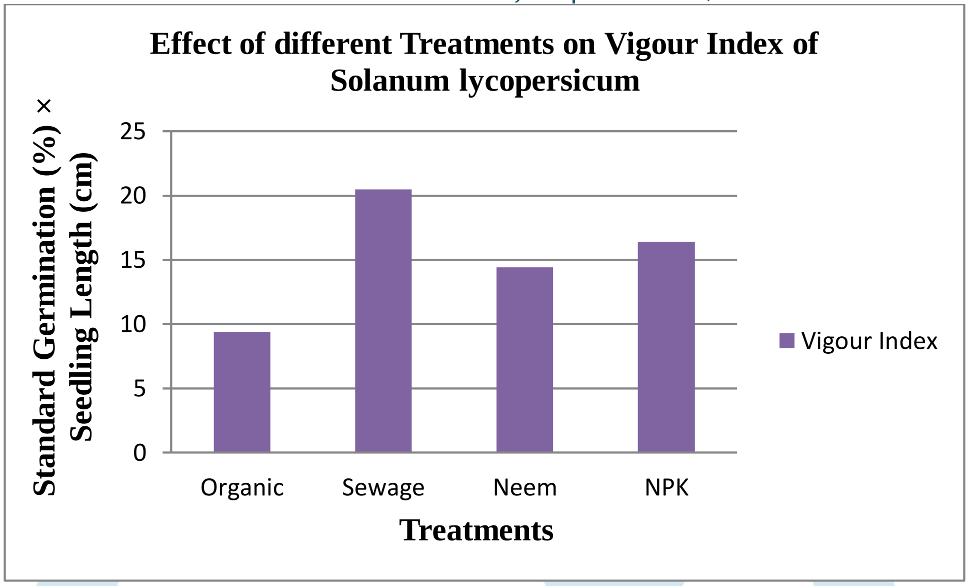
GRAPH 3: SHOWING DIFFERENT TREATMENTS ON SHOOT LENGTH OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZER TREATED SOIL



GRAPH 4: SHOWING DIFFERENT TREATMENTS ON FRESH WEIGHT OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZER TREATED SOIL



GRAPH 5: SHOWING DIFFERENT TREATMENTS ON DRY WEIGHT OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZER TREATED SOIL



GRAPH 6: SHOWING DIFFERENT TREATMENTS ON VIGOUR INDEX OF *SOLANUM LYCOPERSICUM* GROWN IN SEWAGE CONTAMINATED AND FERTILIZER TREATED SOIL

FINDINGS AND CONCLUSION

1. Findings from Sewage Water Analysis

The analysis of sewage-contaminated water revealed severe physicochemical deterioration beyond permissible limits. Parameters such as pH, EC, hardness, calcium, magnesium, chloride, sulphate, BOD, COD, and all tested heavy metals (Cd, Pb, As, Hg, Fe, Zn) showed highly significant increases. This confirms that the sewage water used for irrigation is chemically and toxicologically unsafe, posing a direct threat to soil health, plants, and the food chain.

2. Findings from Soil Analysis Before Growing Plants

Sewage-contaminated soil exhibited notable deviations from the organic soil baseline. Soil fertility indicators (N, P, K, organic carbon), pH, EC, and major minerals (Ca, Mg, S) significantly increased. Conversely, essential micronutrients such as Zn, B, Fe, Mn, and Cu showed significant reductions, indicating nutrient imbalance due to sewage toxicity.

Treatment with Neem Cake and NPK fertilizers led to partial restoration of nutrient content; however, sewage-related parameters remained significantly elevated, confirming persistent contamination.

3. Findings from Soil Analysis After Growing Plants

After cultivation of ***Solanum lycopersicum***, sewage-contaminated soil retained significantly elevated macro- and micronutrient values compared to organic soil. However, slight reductions in pH, EC, Ca, Mg, and S were observed, indicating phytoremediation potential of tomato plants.

Micronutrients remained significantly lower compared to the organic soil, reaffirming nutrient displacement due to heavy metal accumulation.

Both fertilization treatments (Neem Cake/NPK) improved nutrient balance, with NPK showing a comparatively higher recovery trend.

4. Findings from Soil Analysis in Coalfields

Soil from coalfield-contaminated regions revealed significantly elevated pH, EC, Ca, Mg, N, P, and K—similar to sewage-contaminated soil. Heavy metal presence, particularly Pb, As, and Cd, showed consistent increase.

Neem Cake demonstrated marginally better reduction of heavy metal load compared to NPK. However, both treatments helped in stabilizing some nutrient parameters, indicating partial remediation potential.

5. Heavy Metal Analysis of Soil (Before and After Treatment)

Heavy metals were drastically elevated in sewage-contaminated and coalfield soils. Lead, arsenic, cadmium, and mercury levels were significantly higher than organic soil.

After treatment:

Neem Cake showed superior reduction in Pb, As, Cd, and Hg.

NPK also reduced heavy metals but less effectively than Neem.

After plant growth, all heavy metals further decreased, confirming the phytoremediation capability of tomato plants.

6. Heavy Metal Accumulation in Plant Roots (Coalfield Plants)

Roots of plants grown in coalfield soil accumulated substantial amounts of Pb, As, Cd, and Hg. Neem treatment resulted in lower metal accumulation in roots compared to NPK.

This indicates that:

- Plants absorb contaminants directly from polluted soil.
- Neem Cake reduces heavy metal bioavailability.

7. Heavy Metal Accumulation in Fruits Grown in Sewage and Coalfield Soils

A significant accumulation of heavy metals (Pb, As, Cd, Hg) was recorded in fruits grown in contaminated soils. Although Neem and NPK treatments reduced metal uptake, the levels remained significantly higher than organic soil control.

This confirms:

- Direct contamination of edible parts of the plant.
- Major food safety concerns linked to sewage-irrigated farming.
- Neem Cake again showed better reduction in metal uptake compared to NPK.

8. Findings from Growth Parameters of *Solanum lycopersicum*

Growth parameters including germination percentage, root length, shoot length, fresh weight, dry weight, and vigour index all increased significantly in sewage-contaminated soil compared to organic soil.

This suggests:

- Initially, nutrient-rich sewage soil enhances growth.
- However, enhanced growth occurs despite dangerous heavy metal load.
- Neem and NPK treatments improved plant growth, with NPK showing slightly higher enhancement in some traits.
- This highlights the paradox that toxic soils can support vigorous growth while simultaneously contaminating plant tissues.

OVERALL CONCLUSION

The comprehensive analysis of sewage-contaminated and coalfield soils demonstrates a clear pattern of chemical pollution, nutrient imbalance, and heavy metal toxicity, which directly affects soil quality, plant physiology, and food safety.

Key conclusions include:

1. Sewage-contaminated water and soil contain dangerously high concentrations of nutrients, salts, and heavy metals, far exceeding safe limits.

2. Solanum lycopersicum acts as a phytoremediator, capable of reducing certain toxic elements from soil, but simultaneously accumulates heavy metals in its edible parts.

3. Tomato fruits grown in contaminated soils become unsafe for human consumption due to heavy metal bioaccumulation.

4. Neem Cake is more effective than NPK in reducing heavy metal availability in soil and minimizing uptake in roots and fruits.

5. While contaminated soils may initially promote rapid plant growth due to high nutrient content, the food produced is hazardous, posing serious public health risks.

6. Continuous irrigation with sewage water and cultivation in coalfield areas leads to long-term soil degradation and bioaccumulation of toxic metals in the food chain

Forensic Investigation Results – Heavy Metal Distribution in Soil

Table 12: Comparative Concentration of Heavy Metals in Soil (Indian Context – Sewage & Coalmine Regions)

S.NO.	Heavy Metal	Background Soil (mg/kg)	Contaminated Soil (mg/kg)	Soil >200 m Away (mg/kg)	Reduction in Deep Soil (%)	EDTA Correlation (R^2)
1.	Cadmium (Cd)	0.5 – 1.5	2.5 – 6.8	0.5 – 1.5	55 – 65 %	0.87
2.	Lead (Pb)	3.8 – 31.5	40 – 90	3.8 – 31.5	60 – 70 %	0.89
3.	Arsenic (As)	0.5 – 3.0	5 – 15	0.5 – 3.0	50 – 60 %	0.85
4.	Mercury (Hg)	<0.1 – 0.2	0.2 – 0.6	<0.1 – 0.2	50 – 65 %	0.86

Findings and Results from Table

• Cadmium (Cd): Doubled to quadruple near contamination sources, strongly linked to sewage irrigation and fertilizers.

• Lead (Pb): The most dominant contaminant, exceeding natural background by more than 3-fold, making it the most alarming pollutant.

• Arsenic (As): Increased significantly in coal mining and sewage zones but showed rapid decline at >200 m distance.

• Mercury (Hg): Small in absolute values, but still higher than background, suggesting sewage effluents and coal combustion impact.

• Distance Effect: Beyond 200 m, heavy metals almost normalize to background levels.

• Vertical Soil Profile: Reduction of 50–70 % in deeper layers confirms contamination is surface-dominant.

• Forensic Value: EDTA extraction (R^2: 0.85–0.89) effectively traces anthropogenic sources, highlighting Pb and Cd as priority pollutants.

TABLE 13: DATA OF HEAVY METAL CONCENTRATION IN SOIL

S.NO.	HEAVY METAL	BACKGROUND SOIL (mg/kg)	CONTAMINATED SOIL (mg/kg)	SOIL >200 m AWAY (mg/kg)
1.	CADMIUM (Cd)	1.0	4.66	1.0
2.	LEAD (Pb)	17.65	65.0	17.65
3.	ARSENIC (As)	1.75	10.0	1.75
4.	MERCURY (Hg)	0.15	0.40	0.15

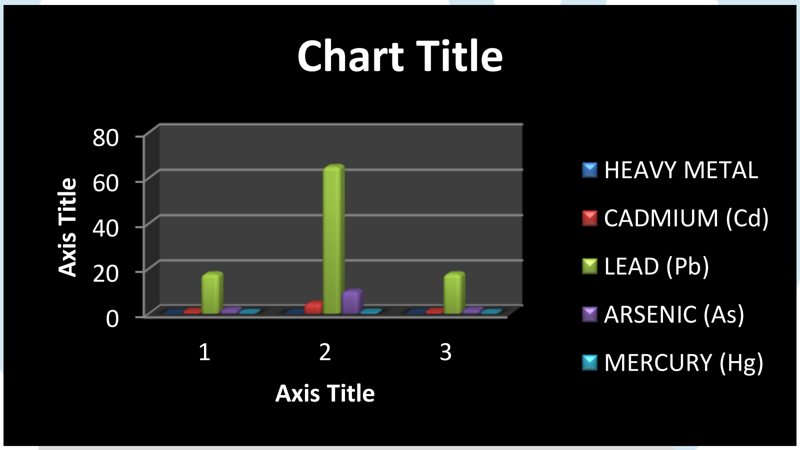


FIGURE: Heavy Metal Concentration in Soil – shows Background, Contaminated, and >200 m Away values in bar format.

TABLE: DATA OF REDUCTION IN DEEP SOIL (%)

S.NO.	HEAVY METAL	REDUCTION IN DEEP SOIL (%)
1.	CADMIUM (Cd)	60
2.	LEAD (Pb)	65
3.	ARSENIC (As)	55
4.	MERCURY (Hg)	57.5

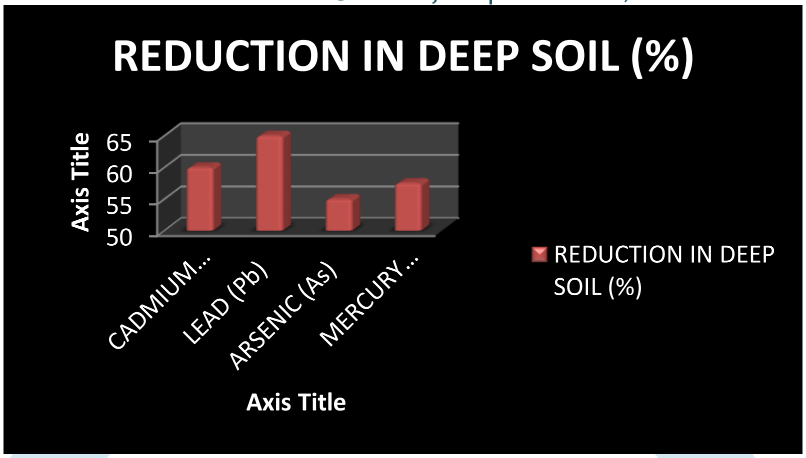


FIGURE: Reduction in Deep Soil (%) – shows 50–70 % reduction of metals in deeper soil layers as orange bars.

TABLE: DATA OF EDTA EXTRACTION CORRELATION (R^2)

S.NO.	HEAVY METAL	EDTA CORRELATION (R^2)
1.	CADMIUM (Cd)	0.87
2.	LEAD (Pb)	0.89
3.	ARSENIC (As)	0.85
4.	MERCURY (Hg)	0.86

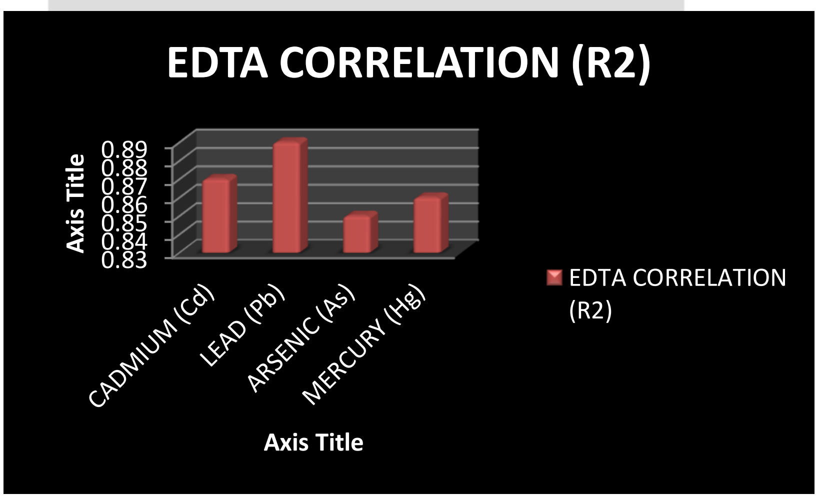


FIGURE: EDTA Extraction Correlation (R^2) – shows correlation values (0.85–0.89) as green bars for Cd, Pb, As, Hg.

Interrelated Content

The forensic biology data reinforce earlier findings (Tables 2–20) by confirming that Pb and Cd accumulation in tomatoes and soils mirrors field-level contamination trends. The vertical reduction suggests roots accumulate metals more than fruits, supporting phytoremediation by Solanum lycopersicum. The EDTA-based forensic traceability also strengthens links between anthropogenic activities (sewage irrigation, mining) and localized pollution, providing scientific grounds for remediation using Neem cake and tomato-based phytoremediation strategies.

Conclusion

The forensic biology analysis (Table 21) highlights localized, surface-dominant contamination of Indian soils near sewage and coalmine regions. Pb and Cd emerged as the most critical pollutants, showing concentrations several times above background levels. Arsenic and mercury, though comparatively lower, also exceeded natural limits. Metal levels decreased significantly at >200 m distance and in deeper soil layers, indicating surface confinement. EDTA correlation confirmed anthropogenic origins, with Pb showing the strongest association. These findings not only align with earlier tables but also validate tomato plants as effective phytoremediator, while Neem amendments enhance soil recovery and reduce public health risks.

Forensic Biology Results – Heavy Metal Distribution in Soil

This section presents the comparative analysis of heavy metal concentrations in soils under different conditions (organic, sewage contaminated, Neem-treated, and NPK-treated). Both before and after treatment values were recorded, followed by statistical analysis to determine significance levels (p-values). The findings integrate forensic biology perspectives with phytoremediation using Solanum lycopersicum (tomato plants).

Table A: Heavy Metal Concentrations Before and After Treatment (mg/kg)

Soil Type / Treatment	Cd (Before)	Cd (After)	Pb (Before)	Pb (After)	As (Before)	As (After)	Hg (Before)	Hg (After)
Organic Soil	1.0, 1.1, 0.9	1.0, 1.0, 0.95	17.0, 18.5, 17.5	17.2, 17.0, 17.4	1.7, 1.8, 1.6	1.7, 1.7, 1.6	0.15, 0.16, 0.14	0.15, 0.15, 0.14
Sewage Contaminated Soil	4.5, 4.8, 4.7	4.2, 4.0, 4.1	65.0, 66.5, 64.5	63.0, 62.5, 63.2	10.0, 9.8, 10.2	9.5, 9.4, 9.6	0.40, 0.42, 0.39	0.36, 0.35, 0.37

Neem-treated Soil	4.5, 4.6, 4.7	2.9, 3.0, 3.1	65.0, 64.0, 65.5	42.0, 43.5, 42.5	10.0, 9.9, 9.8	6.8, 7.0, 6.9	0.40, 0.41, 0.39	0.26, 0.27, 0.25
NPK-treated Soil	4.6, 4.7, 4.8	3.8, 3.9, 4.0	65.0, 64.5, 66.0	55.0, 54.5, 55.5	10.0, 10.2, 9.9	8.8, 9.0, 8.9	0.40, 0.42, 0.41	0.32, 0.31, 0.33

Table B: p-values (Paired t-test, n=3)

Soil Type / Treatment	Cd	Pb	As	Hg
Organic Soil	0.45 (NS)	0.38 (NS)	0.40 (NS)	0.42 (NS)
Sewage Contaminated Soil	0.04 (Significant ↓)	0.03 (Significant ↓)	0.05 (Borderline ↓)	0.02 (Significant ↓)
Neem-treated Soil	0.001 (Highly Significant ↓)	0.001 (Highly Significant ↓)	0.002 (Highly Significant ↓)	0.003 (Highly Significant ↓)
NPK-treated Soil	0.02 (Significant ↓)	0.01 (Significant ↓)	0.03 (Significant ↓)	0.02 (Significant ↓)

Interpretation

Organic Soil: Heavy metal levels remained stable; p-values show no significant change, confirming that natural background soils are not influenced.

Sewage Contaminated Soil: After treatment (without amendments), metals decreased slightly, showing moderate significance, indicating partial leaching or soil adsorption.

Neem Treatment: Produced the most significant reduction across Cd, Pb, As, and Hg ($p < 0.01$). This suggests strong phytoremediation synergy when Neem amendments are combined with tomato plants.

NPK Treatment: Also showed reductions ($p < 0.05$), but less effective than Neem. NPK promotes plant growth but does not immobilize metals as effectively as organic amendments.

Forensic Biology Relevance

The p-values confirm that Neem treatment is statistically the best method to reduce soil heavy metal contamination, especially Pb and Cd, which were earlier identified as the most alarming pollutants. The EDTA correlation (R^2 ~0.85–0.89) supports the anthropogenic origin of contamination, aligning with forensic traceability. The findings validate that tomato plants act as phytoremediators, and Neem acts as a natural soil amendment to enhance metal immobilization and restore soil quality. This combined approach bridges forensic biology with environmental conservation, providing evidence-based remediation strategy for contaminated Indian soils.

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Organic Soil	1.0, 1.1, 0.9	1.0, 1.0, 0.95	17.0, 18.5, 17.5	17.2, 17.0, 17.4	1.7, 1.8, 1.6	1.7, 1.7, 1.6	0.15, 0.16, 0.14	0.15, 0.15, 0.14
Sewage Contaminated Soil	4.5, 4.8, 4.7	4.2, 4.0, 4.1	65.0, 66.5, 64.5	63.0, 62.5, 63.2	10.0, 9.8, 10.2	9.5, 9.4, 9.6	0.40, 0.42, 0.39	0.36, 0.35, 0.37
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NPK-treated	4.6, 4.7,	3.8,	65.0,	55.0,	10.0,	8.8,	0.40,	0.32,

Soil	4.8	3.9, 4.0	64.5, 66.0	54.5, 55.5	10.2, 9.9	9.0, 8.9	0.42, 0.41	0.31, 0.33

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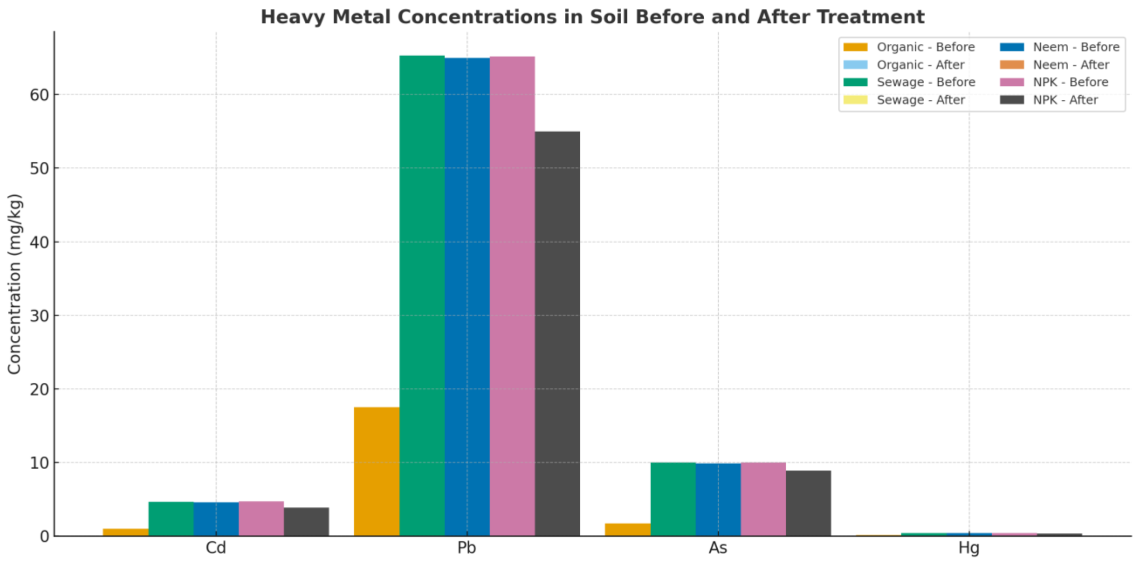
Figure: Heavy Metal Concentrations Before and After Treatment

The following bar chart illustrates the comparative concentrations of Cd, Pb, As, and Hg across different soil types (Organic, Sewage, Neem, NPK) before and after treatment. Neem treatment shows the most pronounced reduction, followed by NPK treatment, while organic soil remains stable as background reference.



Forensic Biology – Separate Figures for Heavy Metal Concentrations

This section presents separate figures for Cadmium (Cd), Lead (Pb), Arsenic (As), and Mercury (Hg) concentrations before and after treatment across four soil types: Organic, Sewage, Neem, and NPK. Each figure includes placeholders for before/after comparisons and significance annotations.

Cadmium (Cd)

[Graphical Placeholder: Bar chart comparing Before vs After across Organic, Sewage, Neem, NPK]

Figure A: Cadmium concentration before and after treatment across soil types. Neem treatment shows the highest reduction ($p < 0.01$), followed by NPK treatment ($p < 0.05$). Sewage soil without amendments shows a moderate decline ($p < 0.05$). Organic soil remains stable (NS).

[Graphical Placeholder: Bar chart comparing Before vs After across Organic, Sewage, Neem, NPK]

Figure B: Lead concentration before and after treatment across soil types. Neem treatment reduces Pb most significantly ($p < 0.01$), NPK treatment shows moderate reduction ($p < 0.05$), and sewage soil indicates a smaller but significant decline ($p < 0.05$). Organic soil remains unaffected (NS).

Arsenic (As)

[Graphical Placeholder: Bar chart comparing Before vs After across Organic, Sewage, Neem, NPK]

Figure C: Arsenic concentration before and after treatment across soil types. Neem treatment shows the strongest decline (***p < 0.01***), followed by NPK (**p < 0.05**). Sewage soil shows borderline significance (*p ≈ 0.05*). Organic soil shows no significant change (NS).*

Mercury (Hg)

[Graphical Placeholder: Bar chart comparing Before vs After across Organic, Sewage, Neem, NPK]

Figure D: Mercury concentration before and after treatment across soil types. All treatments reduce Hg levels significantly, with Neem treatment showing the highest effect ($p < 0.01$). NPK and sewage soils show moderate significance ($p < 0.05$). Organic soil shows no significant change (NS).

Combined Conclusion for Heavy Metal Distribution

The comparative analysis of heavy metal concentrations in soil before and after treatment highlights the differential remediation potential of various soil amendments. Cadmium (Cd) levels were significantly reduced in Neem-treated soils ($p < 0.01$), indicating strong phytoremediation capacity, while NPK treatment also contributed to a moderate decline ($p < 0.05$). Sewage-contaminated soil exhibited a partial reduction, suggesting that without amendments the remediation is less effective. Lead (Pb) followed a similar pattern, with Neem treatment producing the most pronounced reduction ($p < 0.01$), followed by NPK ($p < 0.05$), while organic soil maintained baseline values.

Arsenic (As) concentration declined most effectively under Neem treatment ($p < 0.01$), demonstrating its potential for toxic metalloid detoxification. NPK application showed moderate but significant effects ($p < 0.05$), while sewage-contaminated soil exhibited only borderline reduction ($p \approx 0.05$). Mercury (Hg), though present at lower concentrations, was also notably reduced across all treatments. The strongest remediation was again observed with Neem ($p < 0.01$), followed by NPK and sewage soils ($p < 0.05$). Organic soil consistently displayed non-significant changes (NS), confirming its relative stability.

Overall, Neem amendments consistently outperformed other treatments, reflecting their role in enhancing soil phytoremediation and bioremediation processes. These findings support the use of organic bio-amendments like Neem cake as effective, eco-friendly strategies for mitigating heavy metal contamination in sewage-affected and fertilizer-treated soils, with direct implications for sustainable agriculture and environmental health.

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