

Heavy Metal Contamination and Genotoxic Effects of Gold Mine Effluent on *Allium cepa* L. in Isanlu, Kogi State, Nigeria

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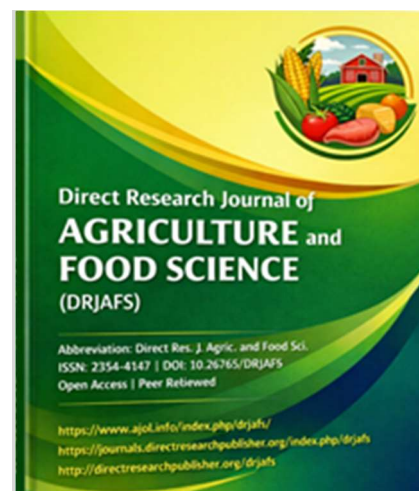
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ABSTRACT

*This study aimed at examining the level of heavy metals as well as the chromosomal abnormalities associated with effluent from the gold mine at Isanlu, Kogi State, Nigeria. Effluent from gold sluicing ore were collected at the point of washing and analyzed for heavy metals. Onion (*Allium cepa*) roots were exposed to varying effluent concentrations (25%, 50%, 75%, and 100%) and analyzed for chromosomal abnormalities while those grown in distilled water serving as control, each treatment had three replicate. The heavy metals concentration in the gold mine wastewater is as follows; Cd (0.02), Cr (0.096), Cu (1.21), Fe (0.25), As (0.015), Ni (0.085), Zn (2.15), Pb (0.06), and Hg (0.0009) The order of abundance of the nine heavy metals in the effluent is as follows: Zn > Cu > Fe > Cr > Ni > Pb > Cd > As > Hg. Four heavy metals (Cd, As, Ni, and Pb) out of the nine analyzed occurred beyond WHO permissible level for effluent discharge. The study revealed that eight chromosomal aberrations which include bridged chromosome, fragmented chromosomes, variant chromosomes, C- mitotic cells, sticky chromosomes, spindle disturbance, binucleate cells, and vacuolated cells were induced in cells treated with different concentrations of the effluent. it was observed that all the concentrations produced negative relative division rates with 25%, 50%, 75% and 100% producing -23.26%, -5.08%, -18.01% and - 20.09% aside from the control. This revealed mito-suppressive effects of the effluent. Evidence from this study strongly indicated that the goldmine effluent is highly contaminated with heavy metals as well as genotoxic and mutagenic at every concentration.*

Keywords: Heavy metals, effluent, genotoxicity, gold mine, *Allium cepa*



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INTRODUCTION

Mining has positively contributed to the economies of many countries (Cisternas. *et al.*, 2022). Kalisz *et al.* (2022) reported that research focus has always concentrated on economic gain of mining but little attention has been paid to sustainable environmental management. The uncontrolled release of contaminated water, or effluent according to Van-Genuchten *et al.* (2022) is a significant issue associated with mining. Substantial release of hazardous heavy metals, particularly cyanide, silver, mercury, and arsenic, were observed during the disposal of effluent from gold mines (Espiritu *et al.*, 2022) These heavy metals are responsible for the contamination of aquatic environments, loss of bio-diversities, as well as neurotoxicity, renal dysfunction, cardiovascular effects in humans (Sing *et al.*, 2022; Agarwal and Darbar, 2022). Tunet *al.* (2020) reported that the major heavy metals associated with gold mines are copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). Artisanal miners use mercury to extract gold, releasing toxic vapours and runoff into the environment in the process. This leads to mercury accumulation in waterways, where it is transformed into methyl mercury and biomagnified through the food chain, posing serious health risks to humans, including neurological damage and organ failure (Krisnayanti *et al.*, 2012; Esdaile and Chalker, 2018; Tinkov *et al.*, 2018). According to Song *et al.* (2019) the environmental impact of this activity is alarming, leading to air, land, and water pollution, habitat destruction, and geological instability.

Given the aforementioned severity of these activities, it is important to monitor and mitigate the effects of mercury pollution. Olusola and Solomon (2018) reported that plant-based bioassays are popular among biological assays because they are quick, affordable, do not require complex laboratory equipment, and have a variety of genetic endpoint. Plants are highly sensitive to even minute variations in the quality of water, and their reactions can serve as early warning indicators of pollutants (Alege *et al.*, 2020). In addition to this, plants and mammals exhibit similar response to mutagens because of the similarities in their chromosomal constituents. *Allium cepa* is one of the higher plant species used for genotoxic and cytotoxicity assessments of pollutants. It has been identified as one of the best bio-indicator for evaluating DNA damage, including chromosomal aberrations and mitotic cycle disruptions induced by contaminants (Alege *et al.*, 2025)

However, studies on heavy metal profiling of gold mine effluent exists (Abdul-Wahib and Marikar, 2012; Rajout *et al.*, 2019; Abdel-Maksoud *et al.*, 2025; Bashirou *et al.*, 2025) very few study integrate this with *Allium cepa* genotoxicity assays for artisanal gold mine effluents offering a novel risk assessment strategy. Furthermore, the favourable chromosomal characteristics of *Allium cepa* such as large chromosomes and reduced diploid

number ($2n = 16$), make it an ideal model for cytotoxic and genotoxic studies (Aruwa *et al.*, 2023). This study therefore aimed to determine the heavy metal composition of the gold mine effluent and evaluate its genotoxic effects on *Allium cepa* root tip cells.

MATERIALS AND METHODS

Description of Study Area

Study area is located in Ilafin, Isanlu the capital of Yagba East LGA, Kogi State, Nigeria. It lies between Latitude $8^{\circ}20'$ N and longitude $5^{\circ}48'$ E of the Greenwich meridian (Figure 1). Farming, small scale mining and fishing are the major occupations of the inhabitants of the town.

Collection of Wastewater Samples

Effluents from gold sluicing ore were collected at the point of washing of the crude gold in the river, into a sterile container, according to the methods outlined by Muhammad *et al.* (2013). Grab techniques was used for gold effluent collection. The sample was immediately transported in an ice box to the department of Biotechnology laboratory, Federal university Lokoja, Kogi State, Nigeria and preserved in a refrigerator below 4°C for chromosomal aberrations study.

Heavy Metal Analysis

The effluent sample were transported in sterilized bottles in ice box to the Laboratory of the Department of Chemistry, Federal University of Technology Akure (FUTA) for heavy metal analysis. For the analysis, the effluent samples were filtered using Whatman filter paper (Number 1) and preserved with 6N.HNO_3 . Concentrations of Cadmium, Chromium, Copper, Iron, Arsenic, Zinc, Mercury, Nickel, and Lead in the effluent were determined using Atomic Absorption Spectrophotometer (AAS) method described by Muhammad *et al.* (2013). Appropriate drift blanks were taken before the analyses of samples. Average values of three replicates were taken for each determination.

Chromosomal Aberrations Study

Allium cepa (onion) bulbs of fairly equal sizes were gotten from international market Lokoja, Kogi State. For this study, small onion (*Allium cepa*) bulbs weighing between 3.00g and 3.50g and measuring $2.0 \pm 0.2\text{cm}$ in diameter were used per treatment concentration, with five (5) replicates per concentration. Each replicate contains five healthy *Allium cepa* bulb. Control bulbs were similarly treated with distilled water. The dry scaly leaves of the

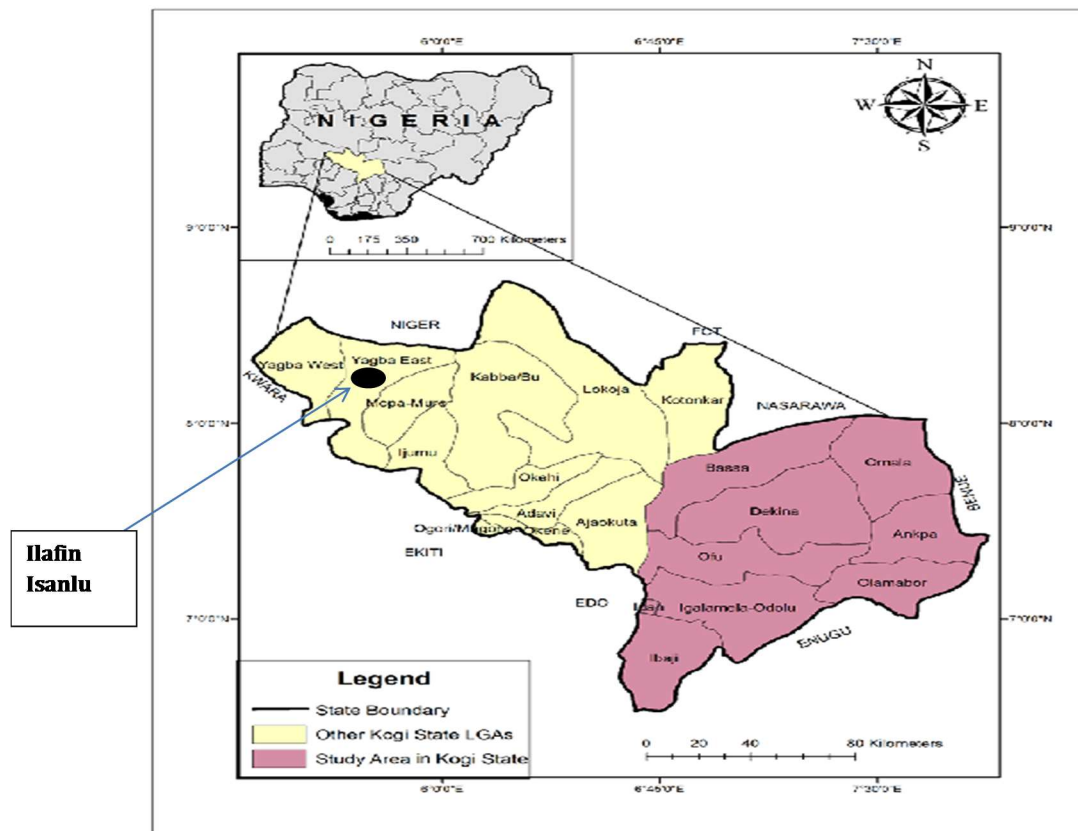


Figure 1: Map of Ilafin-Isanlu Kogi State, Nigeria. Source: Ukabiala *et al.* (2021)

onions were removed while the distilled water in the beakers was allowed to come into contact with the reduced stems in the lower portions of the bulbs. Only bulbs exhibiting uniform root initiation and growth characteristics were selected to minimize biological variability." The onion sets were moved into beakers with varying effluent concentrations (25%, 50%, 75%, and 100%). Roots of each bulb were placed in a 50ml glass beaker containing 30 mL of test solution. Exposure was conducted at room temperature ($25 \pm 2.0^{\circ}\text{C}$), with relative humidity of $60 \pm 10\%$. Bulbs were exposed to a 12 h light/ 12 h dark photoperiod under white fluorescent light at ~ 200 lux intensity. After 24 hours, the roots were taken out of the treatments and thoroughly cleaned. Between 8:00 and 9am, only the roots that were roughly 1cm long were collected into vials. Each treatment was set up using a Complete Randomised Design (CRD), with five replicates. Root tips were fixed, hydrolysed, squashed, and stained using the techniques described by Alege *et al.*, (2020)

Fixation

A fresh solution of 1:3 mixtures of glacial acetic acid and absolute ethanol was used to fix the root tips. The freshly cut root tips were placed into the fixative in vials with clear

labels. After that, the vials were kept in the fridge for 24 hours. The fixative aids in maintaining the cells in their original state.

Hydrolysis

The root tips were removed from the fixatives after 24 hours and properly washed in distilled water according to Wijeyaratne and Wadasinghe (2020) procedures. Hydrolysis is intended to soften the root tips, and this was accomplished by treating the fixed root tips with 1N HCl in a water bath at temperature of 60°C for 10mins.

Squashing and staining

Each hydrolysed root tip was placed on a clean glass slide after being rinsed with distilled water. With a blade, the meristematic tips were removed, and the remaining parts were discarded. Before applying cover slips, the meristems were stained with 1% W/V of aceto-carmine solution prepared by dissolving 1g carmine in 45% glacial acetic acid. Two drops ($\sim 100 \mu\text{L}$) of aceto-caemine were applied to each meristematic root tip on the slide. Staining was performed for 5-7 minutes at room

temperature. Slides were gently warmed over a flame for 2-3 seconds to enhance stain uptake. The flat surface of a cylindrical search pin was used to squash until a cloudy liquid was visible. Extra stain was carefully removed by applying pressure to filter paper. To keep the preparation from drying up, the borders of the cover slip were sealed with limpid fingernail varnish. Five different slides were prepared for chromosomal observation for each treatment.

Chromosome observation

Mitotic cells and chromosomal aberrations were scored under a compound light microscope at 40 x magnifications, 10x eyepiece × 40x objectives for screening and 1000x magnification with oil immersion for confirmation of aberrations. At 400X magnification, photomicrographs of the mitotic stages and abnormal cells were taken. A total of 500 cells per treatment were scored. Approximately 100 cells were examined per slide. Five slides were prepared per replicate to reach the total cell count of 500. The average count per view was recorded. Microscopic fields were selected randomly from well-spread meristematic regions of the root tip. Fields with overlapping cells, poor staining, or mechanical damages were excluded.

Data Analyses

A bar chart was used to represent the averages of the results recorded on the heavy metals in triplicate. Five prepared slides were produced for each treatment, and 10 microscopic views were taken per sample. The total number of dividing cells, the number of cells in each phase of the cell cycle (interphase, prophase, metaphase, anaphase, and telophase), the number of cells in each phase of the cell cycle (telophase), and the number of the various aberrant cells observed were all counted from each microscopic view at 1000X magnification. This was done using a modified procedure of Ping *et al.* (2012). "Data were analyzed using one-way ANOVA in SPSS version 20.0. Means were separated using Fisher's LSD at $P < 0.05$ ". Formulas described by Malode *et al.* (2011) was used to calculate the proportion of cells with chromosome abnormalities, the Mitotic Index (MI), and the Relative Division Rate (RDR) for cells exposed to different concentrations of the wastewater in comparison to the control: Percentage of Aberrant Cells (PAC) was calculated using the formula:

$$(PAC) = \frac{\text{Total number of abnormal cells}}{\text{Total number of cells examined}} \times 100 \quad (1)$$

$$\text{Mitotic Index (MI)} = \frac{\text{Total number of dividing cell}}{\text{Total number of cells examined}} \times 100 \quad (2)$$

The numbers of prophase, metaphase, anaphase and

telophase were summed to represent the total number of dividing cells. The calculation of Relative Division Rate (RDR) was carried out as follow:

$$(RDR) = \frac{\% \text{ of dividing cells in treated root tips} - \% \text{ of dividing cells in control root tips}}{100 - \% \text{ of dividing cells in control root tips}} \times 100 \quad (3)$$

RESULTS

Heavy Metal Concentration of the Wastewater

Table 1 shows the heavy metal composition of goldmine effluent compared with WHO Permissible level. The order of abundance of the nine heavy metals is as follows: Zn > Cu > Fe > Cr > Ni > Pb > Cd > As > Hg. Four (Cd, As, Ni, and Pb) out of the nine analyzed heavy metals occurred beyond the WHO permissible level. Zn, Cu, Fe, Cd, and Hg occurred below the WHO permissible level (Table 1).

Effect of Goldmine Wastewater on Mitotic Cell Division of *Allium cepa*

The effects of gold mine wastewater on *Allium cepa* roots' mitotic cell division are shown in (Table 1). Four of the six mitotic parameters showed significant differences between the treatments examined, according to the study. The quantity of prophase, anaphase, and telophase cells are among the characteristics that differ significantly. The number of interphases and the number of metaphases are the two characteristics that do not differ statistically ($P \geq 0.05$). The table also showed that the total number of treated root tip cells was significantly higher than that of the control. Prophase (11.70) and anaphase (5.50) cell counts were noticeably high at a 50% concentration of the wastewater.

Chromosome Abnormalities Induced by Goldmine Wastewater

Plate I showed the normal stages of mitotic division in *Allium cepa* root tip cells treated with goldmine effluent, while Table 2 and Plate II showed the result of chromosomal aberrations induced by different concentrations of goldmine wastewater on *Allium cepa* root meristems. The study found that eight chromosomal aberrations were induced in the cell exposed to different concentrations of the effluent. The eight chromosomal aberrations recorded in this study are Bridged Chromosome, Fragmented chromosomes, Variant chromosomes, C- mitotic cells, Sticky Chromosomes, Spindle disturbance, Binucleate cells, and Vacuolated Cells. No chromosomal abnormality was recorded in roots treated with distilled water (control). The number of cells with Variant Chromosomes, Sticky Chromosomes, and Spindle disturbance did not show statistical

Table 1: Heavy Metal Concentration of the effluent.

S/N	Heavy metals	GME	WHO	NESREA
1	Cd	0.02	0.005	-
2	Cr	0.096	0.05	-
3	Cu	1.21	2.0	0.1
4	Fe	0.25	0.3	3.0
5	As	0.015	0.05	-
6	Ni	0.085	0.02	-
7	Zn	2.15	3.0	3.0
8	Pb	0.06	0.01	0.05
9	Hg	0.0009	0.001	-

NESREA- National Environmental Standards and Regulations Enforcement Agency in Nigeria maximum permissible limits for effluent

WHO- World Health Organization

GME-Gold mine effluent

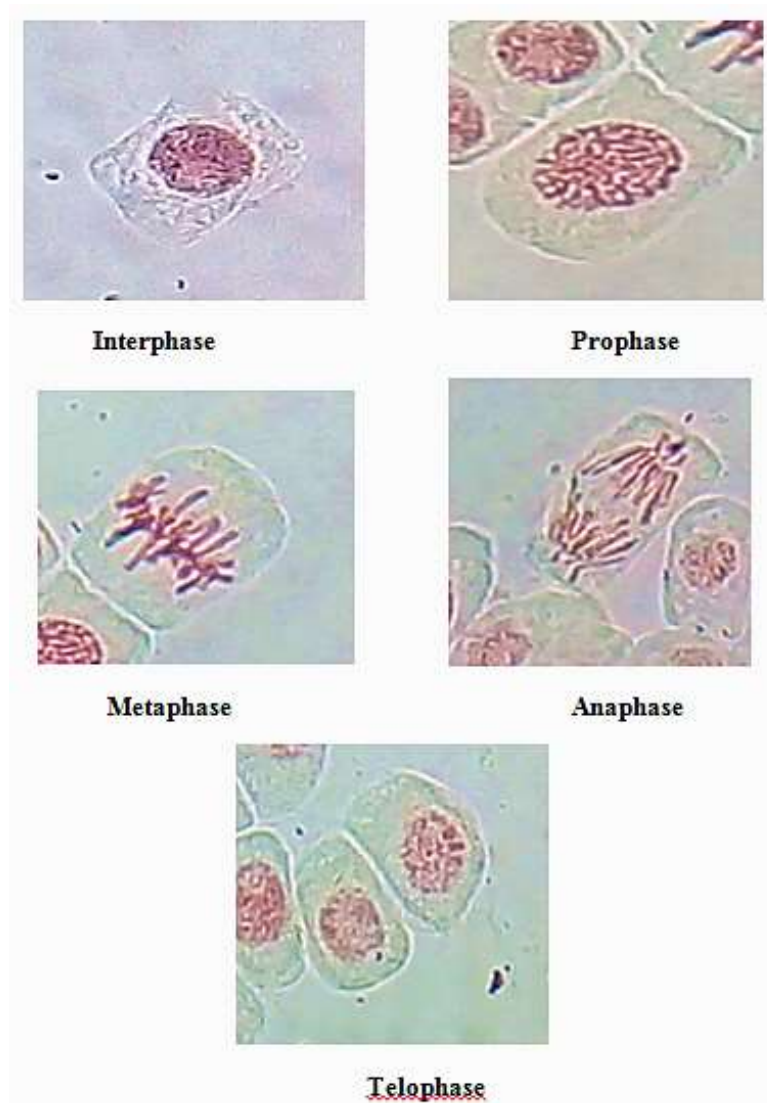


Plate I: Normal stages of mitotic division in *Allium cepa* root tip cells treated with Goldmine Effluent

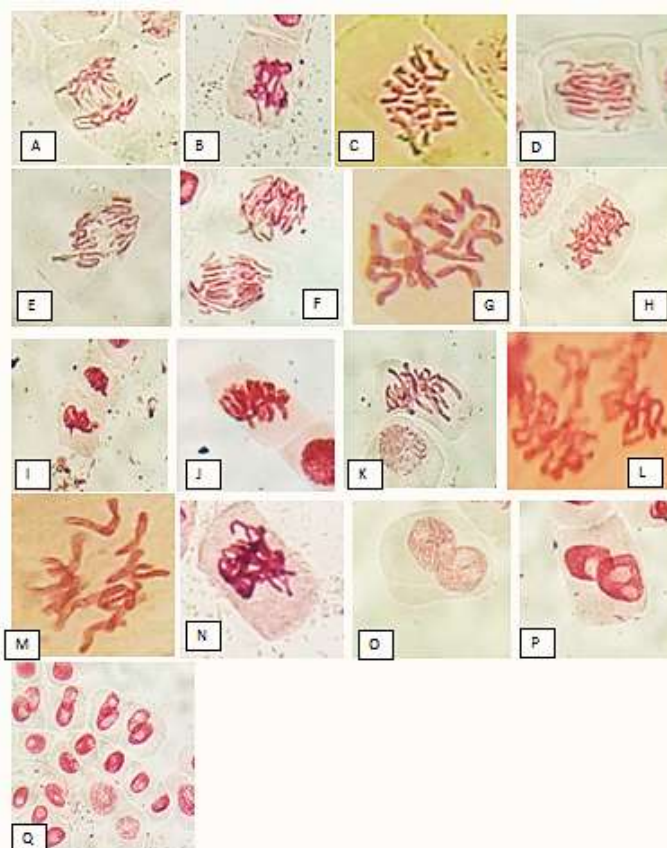
significant differences among the treatments. All the eight chromosome abnormalities were recorded in every concentrations of the wastewater with vacuolated cells significantly induced by the effluent at all concentrations

(Table 3). 50% concentration of the goldmine wastewater significantly caused bridging of chromosomes (0.70), while 75% significantly induced fragmented chromosomes (0.50) and 100% concentration significantly induced

Table 2: Effects of Goldmine Wastewater on Mitotic Cell Division of *Allium cepa*.

Conc.	Average Number of Cells per View	Number of Interphase Cell	Number of Prophase Cell	Number of Metaphase Cell	Number of Anaphase Cell	Number of Telophase Cell
Control	49.30 ^b	30.60	7.30 ^b	5.50	4.00 ^{ab}	1.90 ^a
25%	66.00 ^a	33.50	6.10 ^b	4.00	3.80 ^{ab}	1.60 ^a
50%	66.70 ^a	30.20	11.70 ^a	5.00	5.50 ^a	1.00 ^{ab}
75%	61.30 ^a	30.80	9.00 ^{ab}	4.10	2.90 ^b	0.40 ^b
100%	60.50 ^a	31.50	6.30 ^b	4.70	2.80 ^b	1.60 ^a
LSD Value	8.99	NS	3.62	NS	2.08	1.27

Means with the same superscript along the same column are not significantly different at $P \leq 0.05$

**Plate II:** *Allium cepa* root tip showing aberrant cells induced by Goldmine Effluent

KEY:

A - B: Bridged Chromosomes, C: Fragmented Chromosomes, D - F: Variant Chromosomes, G: C- Mitosis, H - J: Sticky Chromosomes, K - N: Spindle Disturbance; O - P: Binucleate Cells, Q: Vacuolated Cells.

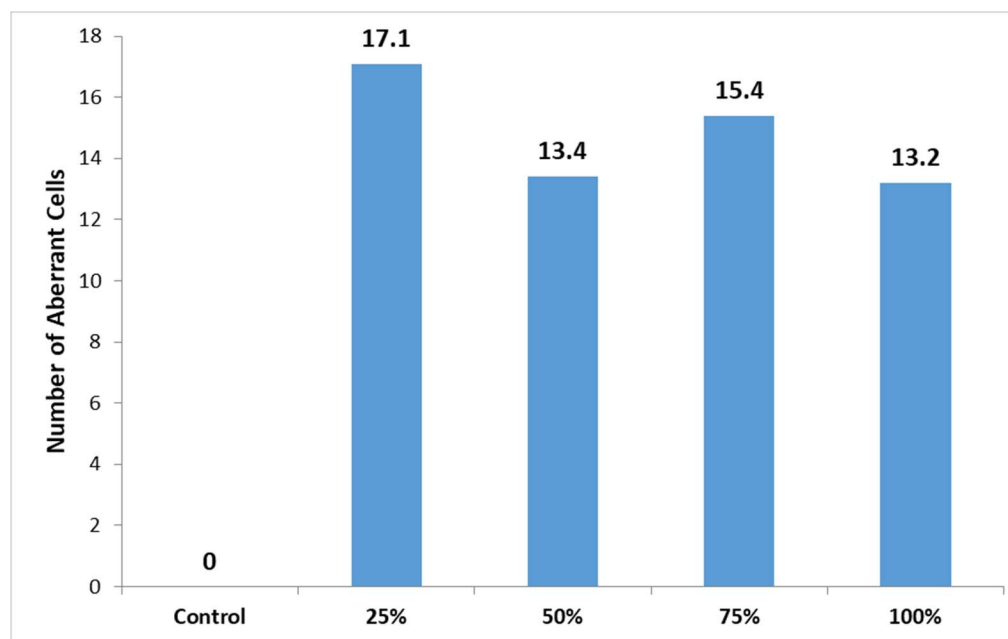
Table 3: Chromosome Abnormalities Induced by Goldmine Effluent.

Conc. %	Number of Bridged Chromosomes	Number of Fragmented Cells	Number of Variant Chromosomes	Number of C-mitotic Cells	Number of Sticky Chromosomes	Number of Cells with Spindle Disturbance	Number of Binucleated Cells	Number of Vacuolated Cells
Control (distilled water)	0.00 ^b	0.00 ^b	0.00	0.00 ^b	0.00	0.00	0.00 ^b	0.00 ^b
25	0.60 ^{ab}	0.30 ^{ab}	0.30	0.20 ^{ab}	0.60	0.20	0.70 ^a	14.20 ^a
50	0.70 ^a	0.10 ^{ab}	0.10	0.10 ^{ab}	0.40	0.30	0.20 ^{ab}	11.50 ^a
75	0.20 ^{ab}	0.50 ^a	0.00	0.30 ^{ab}	0.30	0.10	0.60 ^a	13.40 ^a
100	0.40 ^{ab}	0.30 ^{ab}	0.30	0.40 ^a	0.10	0.30	0.30 ^{ab}	11.10 ^a
LSD Value	0.67	0.48	NS	0.40	NS	NS	0.60	6.76

•Means with the same superscript along the same column are not significantly different at $P \leq 0.05$

Key:

•NS – Not Significant at $P \geq 0.05$

**Figure 2:** Number of Aberrant Cells Induced by Goldmine Effluent

C-mitosis in the treated root tip cells. Figure 2 represent the number aberrant induced by the gold mine wastewater. 25% concentration of the effluent induced the highest total number of aberrant cells

of 17.10 while the least number of chromosome abnormality treated onion roots tips was recorded in 100% of the wastewater (13.20). 50% and 75% concentrations of goldmine effluent induced 13.40

and 15.30 chromosome aberrations respectively while no aberrant cell was recorded in the untreated *Allium cepa* root meristem.

Table 4: Cell Division Frequencies of *Allium cepa* Root Tip Cells Treated with Goldmine Effluent.

Concentrations %	Percentage of Aberrant Cells (%)	Mitotic Index (%)	Relative Division Rate (%)
Control	0	37.93	-
25	25.91	23.49	-23.26
50	20.08	4.78	-5.08
75	25.55	26.75	-18.01
100	21.82	25.46	-20.09

Cell Division Frequencies of *Allium cepa* Root Tip Cells Treated with Goldmine Effluent

Table 4 represents the percentages of aberrant cells, Mitotic Index and relative division rate in the roots of *Allium cepa* treated with different concentrations of goldmine effluent and the control. Roots treated with distilled water (control) did not produce any aberrant cells. Control had mitotic index of 37.93% which is higher than the mitotic indices of 25% (25.91%), 50% (20.08), 75% (25.55%) and 100% concentrations (22.82%). Similarly, when the rates of cell division in the different concentrations of goldmine effluent were compared with control all the concentrations considered produced negative relative division rates with 25%, 50%, 75% and 100% producing -23.26%, -5.08%, -18.01% and -20.09% respectively.

DISCUSSION

Gold mining has been a significant contributor to the growth and sustenance of the economies of many countries (Rajput *et al.*, 2019). According to them, the generation and release of toxic waste products during the mining process have profound impacts on the ecosystem. Masaka *et al.* (2017) stated that heavy metal pollution from gold mines can cause severe environmental issues.

Heavy Metal Status of the Wastewater

The occurrence of Cd, As, Ni, and Pb beyond WHO permissible level in the goldmine effluent indicates the toxic nature of the wastewater. The release of heavy metals into the environment can lead to soil and water pollution, which can have adverse effects on human health and biota. Singh *et al.* (2022) reported that heavy metal pollution from gold mines can cause widespread contamination of the ecosystem, and higher concentrations of these heavy metals above permissible levels could have deleterious effects on human health and biota. The occurrence of Cd, As, Ni, and Pb beyond WHO permissible level in this study conform to the report of Fashola *et al.* (2016) that goldmine is associated with release of large volume of Ni, Pb and AS. Surprisingly, in this study the Mercury content of the effluent was below the WHO permissible standard despite the fact that Mercury is used by the artisanal miners to amalgamate the

gold and segregate it from fine sand. This finding may be connected with the assertion of Krisnayanti *et al.* (2012) that the residual mercury in the ore gets attached to the gold which will be burnt off and released in form of vapor during purification. This vapour according to them can easily be inhaled by people in the vicinity which is of serious environmental and human health concern.

Genotoxic Nature of Goldmine Effluent

The significant variations observed in the number of cells in prophase, Anaphase and Telophase stages across effluent-treated onion root tip cells indicate that the wastewater is genotoxic to the cells. This genotoxicity according to Dutta *et al.* (2018) can lead to chromosomal abnormalities and other negative effects on growth and development of organisms. This finding also suggests that the effluent has significant effect on the cell cycle and possibly damage the spindle fibers, which are necessary for chromosome segregation. This could lead to an increase in the number of cells that are stuck in prophase or anaphase. This finding is consistent with the report of Tkalec *et al.* (2009). The fact that the goldmine effluent induced bridged chromosomes, fragmented chromosomes, variant chromosomes, C-mitotic cells, sticky chromosomes, spindle disturbance, binucleate cells and vacuolated cells in onion root tip cells further supported the genotoxic nature of the wastewater on the cells. The spindle disturbances induced by the wastewater could have given rise to C-mitotic cells, sticky chromosomes, and binucleate cells. The vacuolated cells observed in the onion root tip cells treated with the effluent indicates that the content could induce programmed cell death or apoptosis. Therefore, the study revealed that the genotoxic effects of goldmine effluent on onion root tip cells can lead to chromosomal abnormalities, cell death, and other adverse effects. Dutta *et al.* (2018) reported that effluents from dye industry induced Chromosome Bridge, fragmented chromosomes, and C-mitosis. The presence of these chromosomal aberrations suggests that the effluent from the gold mine is toxic and could cause damage to the genetic systems.

The significant increase in percentage of chromosomal aberrations in root cells of *Allium cepa* grown with different concentrations of goldmine effluents indicates the mutagenic nature of the effluent. Rajput *et al.* (2019), reported that the presence of chromosomal aberrations in

Allium cepa root meristems can be attributed to the high concentration of heavy metals in the environment. Likewise, the absence of chromosomal aberrations in the roots that were treated with distilled water (control) suggests that the damage was caused solely by the contaminants in the effluent.

Effect of Goldmine Effluent on Cell Division Rate of *Allium cepa* Root Tips

The negative relative division rate in relation to control observed in this study suggests that the rate of cell division was lower in all the groups treated with the wastewater than in the control group. This confirms that the effluent from the gold mine is inhibiting cell division. This study revealed that all the concentrations of the effluent considered in this study induced lower Mitotic Indices (MI) and relative division rate compared to the control which indicates that contents of the effluent had mitosuppressive effects on cell division. This means that the toxic chemicals in the goldmine effluent interfered with the normal process of cell division, leading to a decrease in the number of cells undergoing mitosis. The studies therefore suggest that the wastewater can cause root growth inhibition and a decrease in the mitotic index (MI), which is an indicator of cell division. The reduced mitotic indices in root tips treated with goldmine effluent observed in this study conforms to the report of Aksoy *et al.* (2011) who observed reduction in Mitotic division of onion root tip cells treated with different concentrations of effluent from olive oil and milk industry. In this study, the highest damage was observed in 25% concentration of the effluent, followed by 75% of the effluent concentration. The lowest number of aberrant cells was observed in the roots that were treated with 100% concentration of the effluent. This may be because the cells in the roots were already damaged by the 100% concentration of the effluent that they were unable to sustain any further damage. Aruwa *et al.*, (2023) reported similar findings where 25% concentration of the treatment had the highest chromosome aberrations while negative control had the least aberrations.

Conclusion

Evidence from this study strongly indicated that the goldmine effluent is highly contaminated with heavy metals such as Cd, As, Ni and Pb. It can be concluded that the wastewater is genotoxic and every concentration has been observed to induce chromosome abnormalities. The genotoxic effects of goldmine effluent on onion root tip cells can lead to chromosomal abnormalities and cell death (apoptosis). It is therefore important to undertake responsible gold mining practices to minimize the negative impacts of gold mining on the environment and human health. This study revealed that the practice of washing gold ore slurry in rivers as practiced by the artisanal miners, obviously have negative impacts on environmental

and public health. Against this background, this practice should be discouraged.

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