EFFECT OF MERCURIC CHLORIDE ($HgCl_2$) ON CYTOPLASMIC SHUTTLE STREAMING, MORPHOLOGY AND STRUCTURE OF THE PLASMODIAL SLIME MOLD: *Physarum polycephalum*

Anwar D. Maraqa[1], Rajashri R. Naik[1], Ziad A. Shraideh[2] and Ashok K. Shakya[1,*]

[1]Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan
[2]Faculty of Pharmacy, The University of Jordan, Amman 11942, Jordan;

ABSTRACT

The present work is aimed to study the effects of different concentrations of mercuric chloride on the acellular slime mold *Physarum polycephalum*. The effects of this heavy metal on cytoplasmic shuttle streaming, plasmodial structure, and growth were studied. Mercuric chloride (15-20 μg/ml) induced a direct and irreversible termination of cytoplasmic streaming followed by a contraction of the whole plasmodium, strong blebbing, vacuolization, disintegration and disorganization of plasma membrane. Plasmodial strands of *Physarum* treated with different concentrations of mercuric chloride showed irregular cytoplasmic streaming, vacuolization of endoplasm, blebbing of plasmalemma, and disorganization of internal structure.

KEYWORDS: Mercuric chloride, cytoplasmic shuttle streaming, plasmodial slime mold *Physarum polycephalum*.

1. INTRODUCTION

Nowadays, pollutants affect our environment from many sources such as factories which emit many gases, solid substances and a lot of solutions that may contain heavy metals, such as cadmium, mercury, zinc and lead. Although heavy metals are not released extensively in the environment, they may represent a hazard to human health. These chemical pollutants have increased to a level that reached our soil, food, and even drinking water [1]. Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream. High exposures to inorganic mercury may result in damage to gastrointestinal tract, nervous system, and kidneys. Both inorganic and organic mercury compounds are absorbed through the gastrointestinal tract and affect other systems via this route. However, organic mercury compounds are more readily absorbed via ingestion than inorganic mercury compounds [2, 3]. Mercuric chloride ($HgCl_2$) is one of the inorganic mercury compounds that have toxic effects involving numerous organs and body systems. Mercuric chloride can be absorbed through body skin, ingestion and inhalation. It has a strong affinity toward sulfhydryl groups hence, it inhibits a wide variety of enzymes and protein transport systems. The soluble inorganic compounds of mercury are irritating to the skin and mucous membranes, and this effect is particularly marked with mercuric chloride. Concentrations of 1 to 5 percent cause irritation, vesiculization, and corrosion of the skin. Adsorption from skin and mucous membranes may be sufficient to cause systemic poisoning and some people are particularly susceptible to this effect [4]. Few studies were done concerning the effect of pollution with heavy metals on soil microorganisms. Terayama *et al.* [5] studied the toxicity of heavy metals and insecticides on slime mold *Physarum* polycephlum. Wang *et al.* [6], and Zeng *et al.* [7] discussed the influence of heavy metal pollution on soil microbial biomass and enzyme activities. Also, Skerving [8], Bressler and Goldstein [9] showed that lead is able to inhibit or mimic the actions of calcium, and to interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups). Shraideh [10] showed that treatment of *Physarum polycephalum* with Triethyllead (Concentrations between 20-50 μM), caused irreversible condensation of the plasmodial strands, blebbing of the plasma membrane, vacuolization of the cytoplasm and elongation of the cytoplasmic shuttle streaming period. There is still a need for detailed studies concerning the physiological and ultra-structural effects of mercury compounds on significant microorganisms inhabiting our environment. The acellular slime mold, *Physarum polycephalum*, represents a suitable model for studying the effect of environmental pollutants, i.e., heavy metals like mercury, cadmium, nickel and copper compounds on motility, behaviour, and ultra-structure of living organisms. Acellular slime mold, called myxomycetes, represents a strange group of microorganisms. They show plant and animal-like characteristics.

Physarum polycephalum is characterized by having a phagotrophic somatic phase (phaneroplasmodium), which is a yellowish, creeping and multinucleated mass of protoplasm

* Corresponding author

enveloped by a slimy sheath and differentiated into two main regions known as massive front and a posterior network of connected veins [11]. Microscopic evaluation indicates that these veins consist of ectoplasm (gel) and a flowing endoplasm (sol), which exhibits a rhythmic shuttle streaming. In addition, the motive force in *Physarum* is generated by cytoplasmic actin-myosin interaction similar to that of smooth muscles of higher organisms [12]. The present research concentrates mainly on two objectives: (A) Studying the effect of different concentrations of mercuric chloride on plasmodia of *Physarum polycephalum* with emphasis on cytoplasmic shuttle streaming, growth, and viability; and (B) Investigation of structural and morphological effects of mercuric chloride on phaneroplasmodia of *Physarum polycephalum*.

2. MATERIALS AND METHODS

2.1 Study object

Phaneroplasmodium of *Physarum polycephalum* (Jordanian isolate similar to ATCC 44912) was used in this study.

2.2. Effect of test solutions on cytoplasmic shuttle streaming

The shuttle streaming (protoplasmic streaming) is an energy-requiring process. The rate of protoplasmic streaming can be extremely fast (over 1 mm per second) and the duration of an entire cycle of the shuttle streaming is 1.5-3.0 min. Its rhythmicity is a result of its reverse direction every few minutes. Protoplasmic streaming of phaneroplasmodium of *Physarum polycephalum* (starved on 1.5 % non-nutrient agar media for 24 h) was observed after submersion of plasmodia in a physiological salt solution (PSS) as control for about 10 min. PSS consisted of: 6.0 mM NaCl, 3.0 mM KCl, 1.0 mM $CaCl_2$, 0.1 mM $NaHCO_3$ and 0.5 mM $MgCl_2$. Submerged plasmodium in PSS was observed under a phase contrast microscope (at 100x and 400x magnification). After 5-10 minutes of adaptation (when streaming period was nearly stable), shuttle streaming periods were recorded and followed for about 2 h [13]. The PSS solution was removed and replaced with different concentrations of mercuric chloride (5, 10, 15 and 20 µg/ml). After 5-10 min. submersion, shuttle streaming periods were recorded and followed. For every tested solution, 30 periods were measured, the mean and standard deviation were calculated (Table 2). Recorded values were compared with that of a control plasmodium (streaming and morphology under PSS). Also morphological changes were observed at least for 2 h. Depigmentation, the release of pigments and decolouration of plasmodia was observed by the yellowish coloration of submersion solution (Table 1).

2.3 Effect of test solutions on growth of *Physarum* plasmodium

Corn-agar plates containing media prepared from 6 g rolled oats, 1.0 g D-glucose and 7.5 g agar-agar in 500 ml of distilled water were used to test the growth of plasmodia. The media were sterilized for 20 minutes at 121°C and poured in sterile plates. Petri dishes were prepared including growth media (control) and successive concentrations of mercuric chloride (5, 10, 15 and 20 µg/ml), which were added to the growth medium. Small pieces of *Physarum* plasmodia were allowed to grow on medium surface and growth was observed and sketched at intervals of 2h [13].

2.4. Effect of test solutions on structure of *Physarum* Plasmodial strands

Effects of 10 and 20 µg/ml of mercuric chloride on structural organization of *Physarum polycephalum* plasmodial strands were investigated. A phaneroplasmodium of *Physarum* was divided into three pieces. One piece was submerged under PSS (serving as control), and the other two were submerged in mercuric chloride (10 and 20 µg/ml) for 1 h. Treated plasmodial pieces were processed for microscopy as described by Shraideh and Najjar [14]. Plasmodial strands were fixed in Bouin fixative for 24 h. Tissues were fixed using automated tissue processor, dehydrated, cleared, and finally infiltrated by a hot liquid paraffin wax for sectioning. A ribbon of tissue sections were then obtained on a manual rotary microtome (Spencer 50) at 3 µm thickness. Two baths of 30% ethanol and a hot tap water were used to overcome the folding tendency. Thereafter, tissue sections were loaded on a glass slide meshed with egg albumin, dried, stained with classical haematoxylin and eosin stain (H&E), and finally mounted using distyrene, plasticizer, and xylene (D.P.X.) mountant. Then samples were examined and photographed under a Zeiss photomicroscope I.

2.5. Viability test

Viability of phaneroplasmodium of *Physarum polycephalum* (starved on 1.5 % non-nutrient agar media for 24 h) was studied after treating plasmodia with different concentrations (5, 10, 15 and 20 µg/ml) of mercuric chloride for 1 h. Treated plasmodia were transferred to 1.5% agar-agar plates and kept overnight. Their ability to make phaneroplasmodia was compared to that of control (PSS treated plasmodia).

2.6. Time lapse phase contrast photomicrography

The effect of different concentrations (5, 10 and 15 µg/ml) of mercuric chloride on phaneroplasmodium of *Physarum polycephalum* (starved on 1.5% non-nutrient agar media for 24 h) was followed under Zeiss stereo photomicroscope. Photography was started under PSS (at 0-time) then with the test solutions at 10 min. intervals. All experiments were conducted at a controlled temperature (25±2ºC).

3. RESULTS

Acellular slime molds or myxomycetes represent a strange group of microorganisms. They show plant and animal like characteristics. *Physarum polycephalum* is

characterized by having a phagotrophic somatic phase (phaneroplasmodium): It is a yellowish, creeping; multinucleated mass of protoplasm enveloped by a slimy sheath and differentiated into two main regions (1) A massive front and (2) A posterior network of connected veins. In studying the effect of mercuric chloride on *Physarum polycephalum* plasmodia four criteria were taken into consideration: (1) responses of the whole plasmodium to test solution, (2) effect on shuttle streaming periodicity, (3) effect on viability of plasmodia and (4) effect on plasmodial structural organization.

The effects of different concentrations of mercuric chloride on whole phaneroplasmodia migrating on agar surface were summarized in Table 1 which shows the sequence of events that occurred after treating plasmodia with mercuric chloride. The main effects observed are disruption of cell membrane, condensation of cytoplasm, vacuolization, blebbing and decolouration of plasmodium. Table 2 summarizes the effect of mercuric chloride on the shuttle streaming periodicity. The effect of sub-lethal dose (15 µg/ml) and the lethal dose (20 µg/ml) was investigated on the structure and behaviour of treated phaneroplasmodia. The high concentration of mercuric chloride produce direct irreversible stop of streaming. Low concentration produces elongation in streaming period and vacuolization.

TABLE 1 - Effect of gradient concentrations of mercuric chloride ($HgCl_2$) on *Physarum plasmodia*.

$HgCl_2$ (µg/ml)	Effect
20.0	Direct fixation of plasmodium.
15.0	Direct condensation of the whole plasmodium, release of pigments to the outside and consequent decolouration of plasmodium. In addition to high aggregation of cytoplasm, more vacuolization of cytoplasm and blebbing of plasmalemma.
10.0	Condensation of cytoplasm, aggregation and high degree of vacuolization in addition to slight blebbing of plasmalemma.
5.0	Disruption of thin veins, followed by slight vacuolization and cytoplasmic condensation

TABLE 2 - Effect of $HgCl_2$ (gradient concentrations) on plasmodial shuttle streaming periodicity.

Treatment	Period			Number of plasmodia used N	Observation
	Time in minutes Mean ± SD	Percentage	Number of periods analyzed		
PSS	1.74 ±0.60	100	30	3	Regular streaming, large vacuoles
5.0 (µg/ml)	2.82±0.8	162	30	3	Elongation in streaming period, and little vacuolization
10.0 (µg/ml)	3.63±1.0	208	30	3	Elongation in streaming period, disturbance occurred in large veins, obvious vacuolization and some blebs occurred.
15.0 (µg/ml)	-	-	-	3	Irreversible stop of streaming after few minutes
20.0 (µg/ml)	-	-	-	3	Direct irreversible stop of streaming

Percentage: (Mean of the treated /Mean of the control)×100%

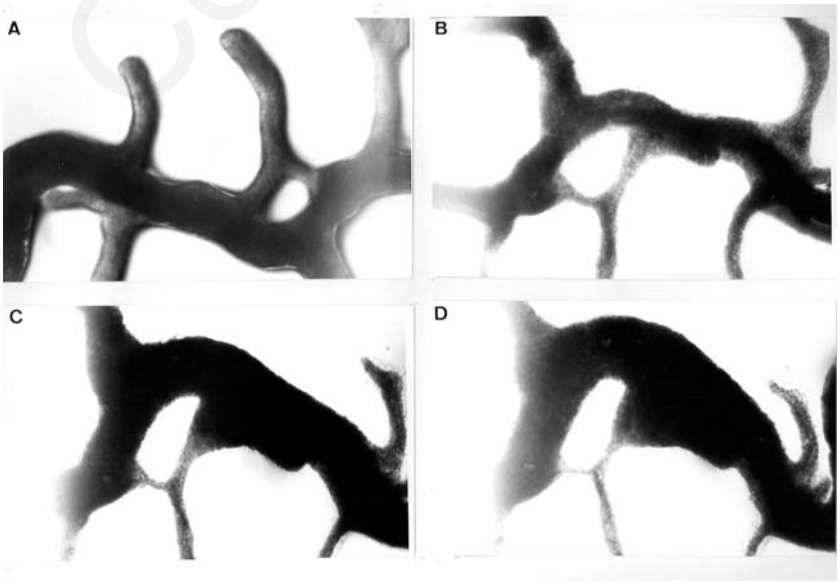

FIGURE 1 - Time lapse contrast photomicrography of *Physarum* plasmodium submerged under 5 µg/ml $HgCl_2$ with 10 min. intervals. (A). At zero time - normal structure of protoplasmic strand was observed. (B) After 10 min. - note slight vacuolization and cytoplasmic condensation. (C) After 20 min. - cytoplasmic condensation. (D) After 30 min. - more condensation and vacuolization of endoplasm was observed. Magnification = 40*X*.

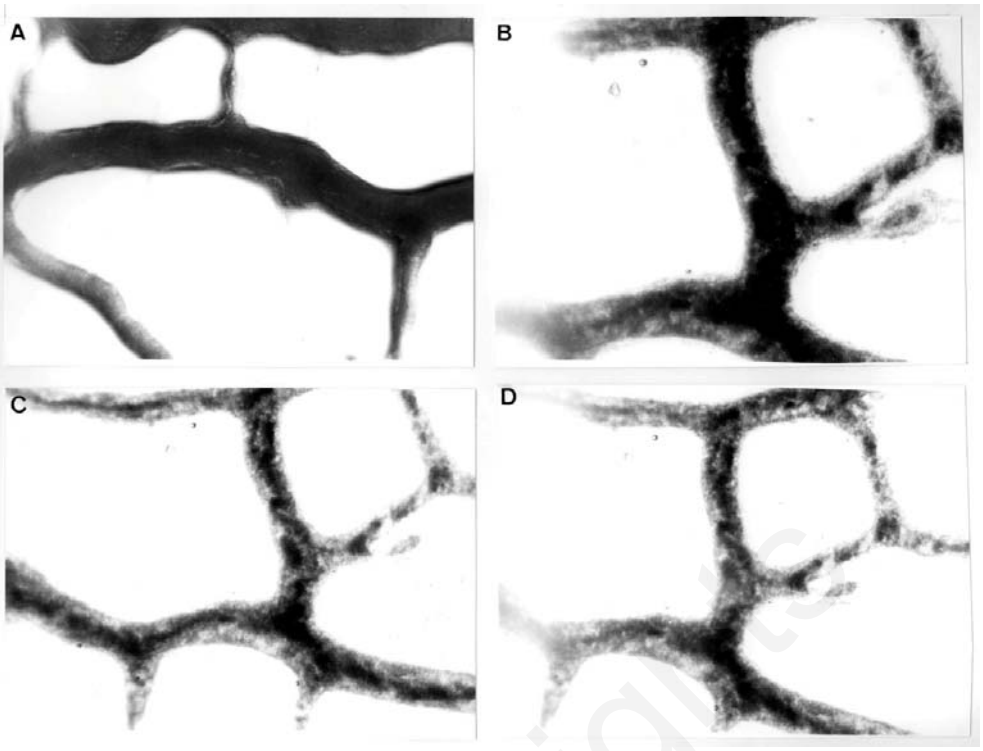

FIGURE 2 - Time lapse phase contrast photomicrography of *Physarum* plasmodium submerged under 10 µg/ml $HgCl_2$ with 10 minutes intervals. (A)At zero time - normal structure of protoplasmic strand was observed. (B) After 10 min. - note condensation of cytoplasm. (C) After 20 min. - more condensation and start of vacuolization was observed. (D) After 30 min. - cytoplasmic aggregates (arrow) and vacuolization (arrow head) was observed. Magnification = 40*X*

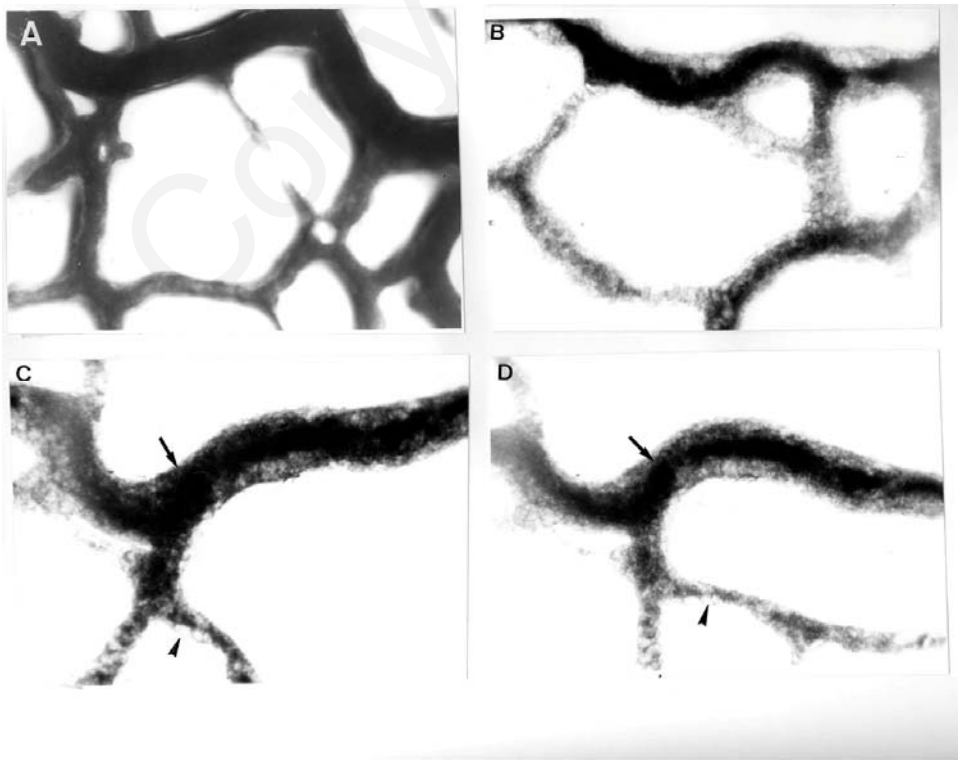

FIGURE 3 - Time lapse phase contrast photomicrography of *Physarum* plasmodium submerged under 15 µg/ml $HgCl_2$ with 10 min. intervals. (A) At zero time - normal structure of protoplasmic strand was observed. (B) After 10 min. - Note start of cytoplasmic condensation (arrow) and vacuolization (arrow head). (C) After 20 min. - formation of cytoplasmic aggregate (arrow), and blebbing of plasmalemma (arrow head) was observed. (D) After 30 min. - more aggregation of cytoplasm (arrow), vacuolization, and blebbing of plasmalemma (arrow head) in addition to depigmentation of plasmodium was seen. Magnification = 40*X*

The viability of the plasmodia was affected by different concentration of mercuric chloride (10, 15 and 20 µg/ml). Plasmodia treated with 10 µg/ml of mercuric chloride showed very weak spreading (compared to that of control). While 15 and 20 µg/ml of mercuric chloride resulted in complete inhibition of spreading of plasmodia; they were unable to make phaneroplasmodia. Effect of the three different concentrations of mercuric chloride on *Physarum* plasmodia was followed under the microscope. High concentration of mercuric chloride (20 µg/ml) produces cytoplasmic condensation and vacuolization after 10 minutes. As time increases there was formation of cytoplasmic aggregates and blebbing of plasmalemma, which increased with time period. Low concentration of mercuric chloride produces minor change in vacuolization and cytoplasmic condensation, which increased with increase in time (Figure 1-3). There was organizational disruption, vacuolization, fragmentation of protoplasm and narrowing of ectoplasm was observed with high concentration of mercuric chloride (Figure 4 and 5).

4. DISCUSSION AND CONCLUSION

This pilot study is conducted first time to observe the effect of environmental contamination especially inorganic mercury on different microorganisms inhabiting our environment. In this study we investigate the effect of different concentration of mercuric chloride to study the different parameters of life of the acellular slime mold *Physarum polycephalum*. The results showed that very low concentration of mercuric chloride (5-20 µg/ml) affected severely the activity, structure and life of the acellular slime mold *Physarum polycephalum*. Disruption of thin veins, slight vacuolization, and cytoplasmic condensation was observed with a very low concentration of mercuric chloride (5 µg/ml). It induced also about 60% elongation in streaming period. Furthermore, 10 µg/ml of mercuric chloride showed 108% elongation of the streaming period, and nearly stopped the migration of the plasmodia. In addition to condensation of cytoplasm and aggregation, high vacuolization was observed. Finally concentrations of 15 µg/ml and higher caused a complete stop of cytoplasmic streaming of the plasmodia. They also affected irreversibly the viability of the plasmodia and prevented the formation of the migrating phaneroplasmodia. From the structural observations we can conclude that mercuric chloride affects the integrity of the biological membranes by making irreversible bond with the different binding sites of enzymes and proteins. The weakening of the membrane led to the observed effects, *for example*, vacuolization of cytoplasm, blebbing of plasmalemma and its disruption. The consequences of disruption of gross organization of plasmodial strands were observed as irregular streaming, stop of migration, cytoplasmic aggregation and finally blebbing of plasmalemma and its disruption. Similar effects were obtained after treatment of *Physarum* plasmodia with triethyl lead chloride [10]. The significance of the work can be understood when we know that *Physarum polycephalum* is a soil-free living microorganism and other

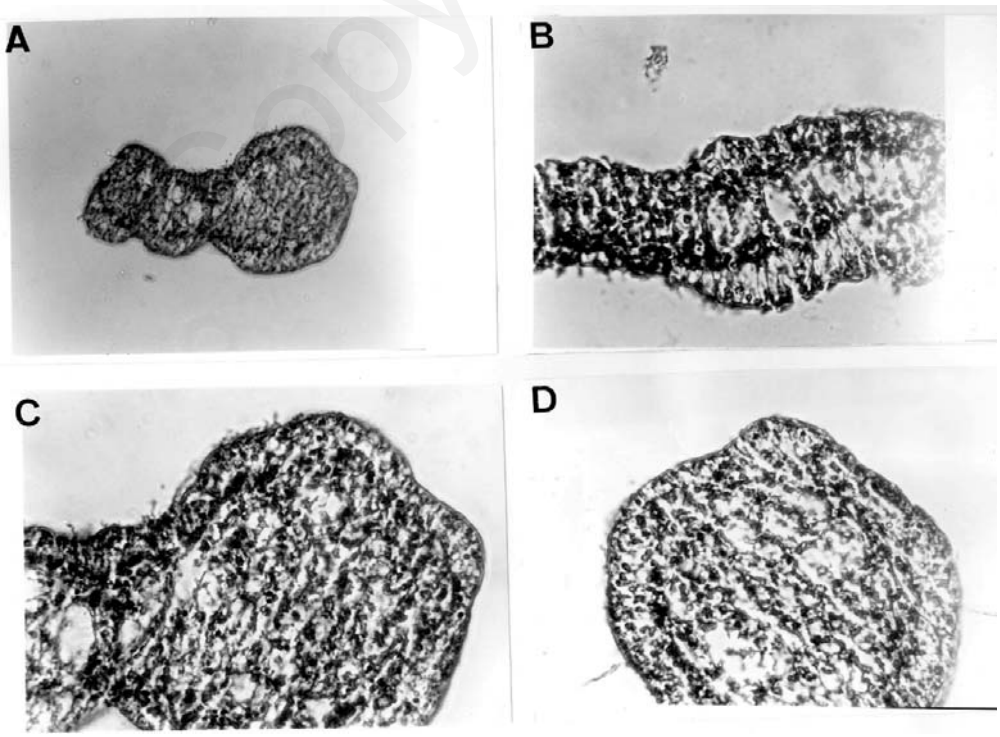

FIGURE 4 - Structure of control *Physarum* protoplasmic strand. (A) X.S. in three fused veins, showing ectoplasm (E), and endoplasm (N) Magnification=114*X*. (B) L.S. showing ectoplasm (E), endoplasm (N), and plasmalemma invagenation (P). Magnification =285*X*. (C) X.S. higher magnification of A. showing ectoplasmic (E) and endoplasmic (N) region, Magnification =340*X*. (D) X.S. as in (C), Magnification = 340*X*.

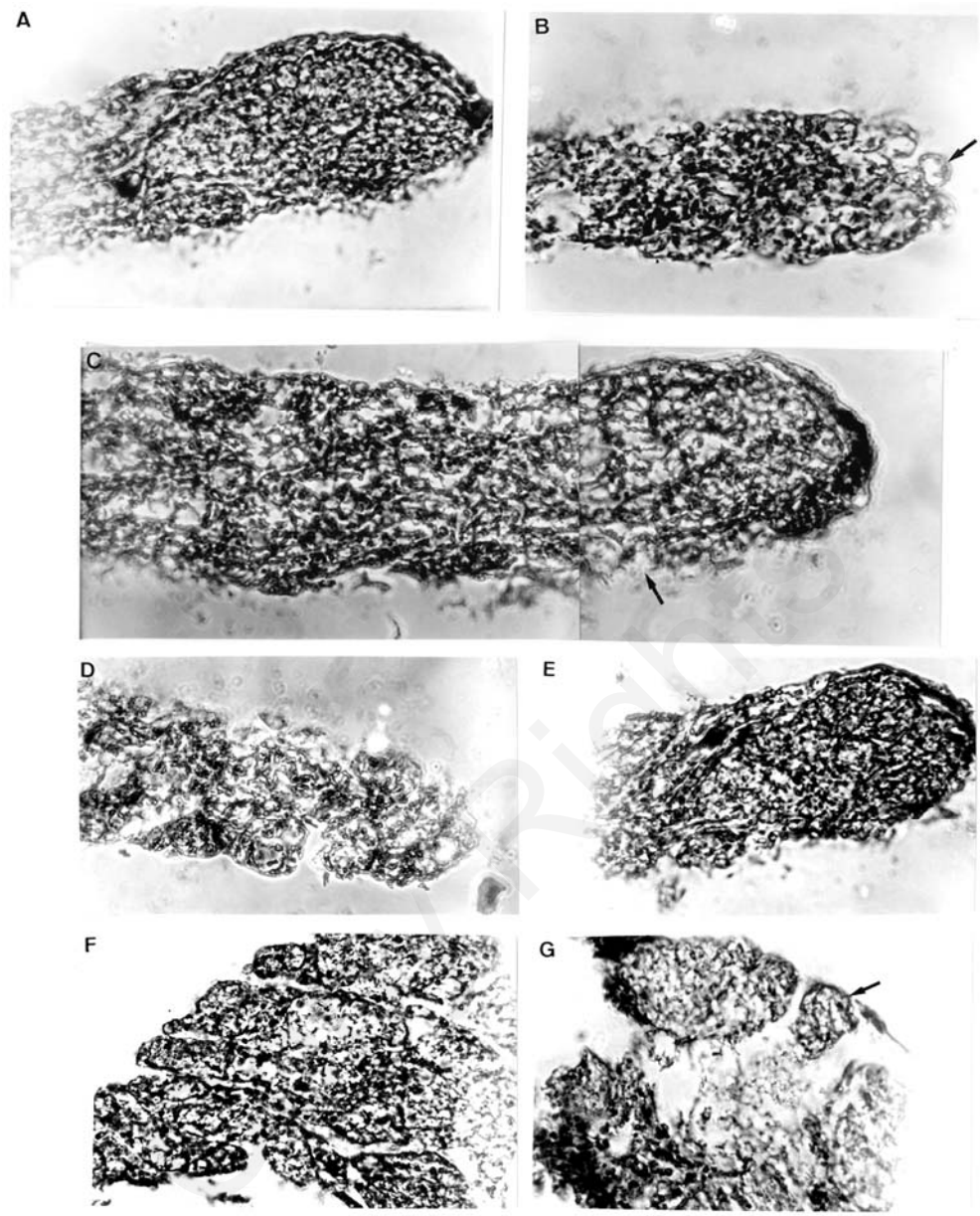

FIGURE 5 - Effect of $HgCl_2$ (20 µg/ml) on structure of *Physarum* plasmodial strand (submersion period was 1h). (A) L.S. in a plasmodial strand treated with 20µg/ml $HgCl_2$ for 1h. Note disruption of organization and very narrow ectoplasm (arrow), Magnification =340*X*. (B). L.S. showing protoplasmic aggregation, and blebbing of plasmalemma (arrow), Magnification=340*X*. (C). L.S. higher magnification showing high vacuolization, disruption of organization, and partial disruption of plasmalemma (arrow). (D) L.S. showing fragmentation of protoplasm and blebbing of plasmalemma (arrow), Magnification =357*X*. (E) L.S. as in A, Magnification=357*X*. (F) X.S showing fragmentation of protoplasm. Magnification =357*X*. (G) X.S. showing severe fragmentation of protoplasm, and formation of blebs (arrow), Magnification =328*X*.

free living microorganisms may be affected similarly. Contamination of environment with heavy metals like mercury will have an undesirable effect on the environmental structure and ecosystem.

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CORRESPONDING AUTHOR

Ashok K. Shakya
Faculty of Pharmacy and Medical Sciences
Al-Ahliyya Amman University
PO Box 263, Amman 19328
JORDAN

E-mail: ashok2811@yahoo.com

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