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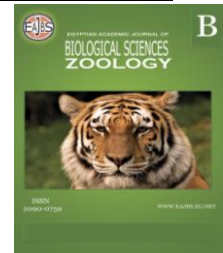


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## Towards Study of UV-C Radiation Effect on Earthworms and Isopods Via Electron Microscopy

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### ABSTRACT

**Background and Objective:** Ultraviolet (UV) radiation is the most significant modifiable risk factor for eye and skin disorders especially skin cancer, atrophy, pigment shifts, and wrinkling in many living organisms. So, this study is based on the detection of the cumulative effects of UV-C radiation on the earthworms (as an alternative model for human skin) and isopods (as an alternative model for the eye) coupled with scanning electron microscopic analysis (SEM). This study was designed for the assessment of human health implications and for the planning of safety measures for human beings.

**Materials and Methods:** Random samples of earthworms and isopods were collected from different areas. Ten earthworms (*Lumbricus castaneus*) and fifteen isopods (*Porcellio laevis*) per set were put in a plastic container and were placed in four groups: control and three UV-C treated groups. The groups were exposed to UV-C for (15 min/day, 30 min/day and 60 min/day) for three consecutive days using a Laminar Flow UV (254 nm). However, the control group was kept under laboratory conditions and without exposure to UV-C. Morphological changes of earthworms and isopods were examined using SEM.

**Results:** Mild changes appeared after exposure of earthworms and isopods to UV-C for 15 min/day. These changes are maximized after 30 min/day. Complete disappearance of the cell junctions in earthworms with sloughed tissue as well accumulation of crusts between junctions of the compound eye in isopods were observed after exposure for 60 min/day.

**Conclusion:** For the first time, SEM analysis was established to detect the influence of UV-C radiation on earthworms and isopods. It was found that prolonged exposure to UV-C radiation induced severe damage and an increase in the mortality rate for both earthworms and isopods.

### INTRODUCTION

The earthworms comprise the dominant soil invertebrate biomass (Kooch and Jalilvand, 2008), which give numerous services to the ecosystem provided to soils (Hoeffner, *et al.*, 2018). Earthworms are considered ecosystem engineers (Kooch and Jalilvand, 2008), the intestines of the earth (Johnson *et al.*, 2002) and keystone species

(Sabrina *et al.*,2009). Earthworms are among the most important soil-dwelling invertebrates that their activity affects both biotic and abiotic soil properties (Van Groenigen *et al.*,2014 and Abd El-Aziz & Bashandy, 2019). More than 3500 species are recognized worldwide and further surveys expected this number to be much greater (Deleporte, 2001 and Hendrix & Bohlen, 2002). Their casts, burrows and related intermediates are a very favorable micro-environment for microbial activity (Hale *et al.*,2005 and Hale &Host, 2005).

Earthworms were used as an alternative model of human skin based on reported similarity to human skin; both contain triene and tetraene sterols (Abd Ellah *et al.*, 2019 and Albro, *et al.*,1997). They were utilized as a model to study the phototoxic effects of UV rays on the skin and as a predictor of environmental risk factors (Albro *et al.*,1997, Hirano &Tamae, 2010 and Misra, *et al.*, 2005). Notably, earthworms are considered a simple, cheap and sensitive animal model for preclinical studies, rather than using the higher laboratory animals (Abd El-Aziz & Bashandy, 2019, Abd Ellah *et al.*, 2019, and Misra, *et al.*, 2005).

The terrestrial isopods (Crustacea: Oniscidea) are one of the most morphologically and ecologically diverse groups of crustaceans including a large number more than 3,710 known species, which are members of the soil fauna and terrestrial isopods (Sfenthourakis & Taiti,2015). Considering tools for the ecotoxicological assessment of metal-contaminated litter and soil, isopods have an important role in the functioning of ecosystems (Bouchon *et al.*,2016). They are useful in the recycling of nutrients, leading to fragmentation of decaying plant material that benefits the soil. Additionally, the feces of isopods are the basis for the formation of soil aggregates and humus, which enlarge its capacity to stock up nutrients (Culliney, 2013). Some facets of its recovery, however, remain enigmatic in nature (Shao *et al.*,2020).

Furthermore, UV radiation was proposed to cause damage to earthworms when they are on the surface of the soil. Earthworms and isopods usually do not expose themselves to sunlight. However, sometimes after heavy rain, earthworms do not go back to the soil and can be found dying in the mud (Quadros, 2010 and Waller& Verdi, 2017). In this analysis, we tried to test this hypothesis (Quadros, 2010 and Waller& Verdi, 2017).

Components of solar radiation are divided into UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm) (Steeger *et al.*, 2001). Owing to the loss of the ozone layer, it has increased the rate of UV solar in recent decades, affecting humans (causing; skin erythema, skin cancer and eye damage), the atmosphere (Ballare *et al.*,2011 and Bornman *et al.*,2015) as well toxicity to soil invertebrates (Gomes *et al.*, 2018). Upon prolonged exposure to UV (UV-B and -C) radiation, major health risks and skin disorders (like atrophy, pigment changes, wrinkling and malignancy) and also causes a lot of damages on phytoplankton, coral, insects, plants and humans were reported by D'Orazio *et al.*(2013) and Herndon *et al.*(2018).On the other hand, real doses of UV radiation were reported to create a considerable pressure and had a substantial negative impact on isopods (Ferreira *et al.*,2016), invertebrates (Marshall *et al.*,1996) and earthworms (Chuang *et al.*,2006 and Edwards &Bohlen, 1996).

Regarding reviewing the literature, only UV-A and UV-B effects on earthworms were studied (Misra, *et al.*, 2005 and Chuang *et al.*,2006). So, we tried and for the first time to investigate the cumulative effects of UV-C radiation on the two species of invertebrates; earthworm, *Lumbricus castaneus* (Savigny, 1826) used as a robust human skin model and isopod, *Porcellio laevis* (Latreille, 1804) used as a model to evaluate the potential effects of UV-C radiation on isopods eyes by using a scanning electron microscope (SEM).

## MATERIALS AND METHODS

### Study Area:

This study was carried out at the laboratory in Zoology Department, Faculty of Science, Assuit University, Assiut Governorate, Egypt (Lying between 27° 14' N and 31° 11' E) from September 2019 until February 2020.

### Samples Collection:

Random samples of earthworms and isopods were collected from seven sites of various cities. Eighty-nine adult earthworms (Clitellate) were selected and one hundred adult isopods were collected and transported to the laboratory. Samples were held in small plastic boxes; the box bottoms were filled with a mixture of sand and potting soil in addition to a wet piece of cotton. Earthworms and isopods were kept under laboratory conditions (25–28°C with 12h light: 12h darkness) and fed daily (earthworms fed with cow manure, leaves and sand; isopods fed with leaves) for one month to acclimatize before the experiments to laboratory conditions. The collected samples were put in four groups: control group without exposure to UV-C and three groups treated with UV-C radiation (one exposed for 15 min/day, one exposed for 30 min/day and one exposed for 60 min/day, the three groups for three successive days).

### Exposure to UV-C Radiation:

The observation protocol for the influence of UV-C radiation with  $\lambda = 254\text{nm}$ . Ten earthworms and fifteen isopods per set were mounted in plastic containers (20 cm\*20 cm) containing 2 mm of buffered saline (0.01 M, pH 7.4) or moist filter paper with buffered saline and exposed to UVR-C using a Laminar Flow UV (Clifton, NJ, USA using three bases of glass germicidal UV lamp wattage 15 ozone free made by Sankyo-Denki, Japan.) at Molecular Cell Biology Laboratory, Department of Zoology, Faculty of Science, Assiut University. For earthworms and isopods, each species has divided into four groups; the control group (Group,1) was kept under laboratory conditions and without exposure to UV-C. While the three groups in each set (earthworm and isopods) Group (2) irradiated for 15 min/day, Group (3) irradiated for 30 min/day and Group (4) irradiated for 60 min/day, each group for three days and three containers replicates for each group.

### Mortality of Earthworms and Isopods Exposed to UV-C:

Daily after exposure to UV-C. When the prostomium in earthworm showed no response to the contact with the probe, and isopods showed no response or movement. In this case, we are sure from the death of the earthworm and isopods. Recorded Death Time.

### Scanning Electron Microscopic Examination:

SEM analysis was carried out at the Electron Microscopy Unit, Assiut University. The entire samples (earthworms and isopods) were put on cover slides and fixed for 1.5 h in a sodium cacodylate buffer with 5% glutaraldehyde, rinsed in distilled water and dehydrated in ethanol. Drying of critical points was done. Samples were installed on stubs, covered with carbon or gold then examined by Joel JSM 35 Scanning Electron Microscope at 20 kV.

### Statistical Analysis:

Triplicate experiments were performed. The significance of differences between the control group and treated three groups were examined by one-way ANOVA using Prism statistical software. A level of significance of  $P < 0.05$  or  $0.01$  was accepted as significant or highly significant, respectively.

## RESULTS

### Earthworms:

The control earthworms (Group,1), kept under laboratory conditions and without exposure to UV-C, showed no evidence of toxicity. The earthworm body was segmented had essential structural functions. They were about 30–70 mm wide and had 82–100 segments. The mouth was in the front end (Fig.1 A&B). The ventral anterior portion of the earthworms revealed an orange clitellum (27:32) (Fig.2, A), which is fond dorsal and secretes a viscous fluid to form a cocoon for its eggs. The main function of clitellum is cocoon formation. Earthworms had parts that helped them in movement. Each section has muscles and bristles so-called setae (Fig.3A& B). The setae helped in anchoring and regulating the worm while passing through the soil. The pair of genital setae was used during mating to help tie two worms together while facing one another in opposite directions. Female genital pores and seminal receptacles were only visible when the worm was in reproductive condition.

Exposure to UV-C for 15 min/day (Group,2), induced different changes in mouth opening including dryness of the skin with formation of an ulcer in the first segment (prostomium) and second segment (peristomium) (Fig.1C), irregular groves and tunnels (Fig.2B) and dryness of the outer layer with mild damage of the setae ends (Fig. 3 C&D). Also, exposure to UV-C for 30 min/day (Group,3), persuaded the disappearance of the cell junctions of the most superficial layer (Fig.1D), dryness and erosions (Fig.2C) as well broken ends of setae (Fig.3E).

Moreover, severe ulcer with complete closure of the mouth opening (Fig.1E), necrosis and desquamation of epidermis (Fig.2D), deep separated transverse grooves and crusts formation on the body and damaged setae (Fig.3 F&G) was observed upon exposure to UV-C for 60 min/day (Group,4).

### Isopods:

Concerning the control isopod (Group,1), s; a large woodlouse was usually about 20 mm long. The isopod body was smoothly glossy and dark surface and was divided into three distinct regions: head (cephalon), thorax (pereon), and abdomen (pleon) (Fig. 4A& B).

Exposure to UV-C for 15 min/day (Group, 2), prompted mild swelling in the head surface (Fig. 4 C). Marked multiple swellings were more obvious after exposure to UV-C for 30 min/day (Group,3), (Fig. 4D). Although, exposure to UV-C for 60 min/day (Group,4), causes severe damage in the head accompanied by complete loss of one eye (Fig. 4E). The eyes were situated front-laterally on the head and its color was brown. The cornea covered the outer surface of the eye and the crystalline cones of the ommatidia. The eye is comprised of about 25 ommatidia. Hexagonal packing of the ommatidia was noticeable through the transparent smooth eye cuticle. The dioptic systems of each ommatidia contained a biconvex corneal lens and a spherical crystalline cone. The cuticular lenses were about 0.1 mm in diameter (Fig.5A) the wall of the body surface of isopod *Porcellio laevis* surrounds the eyes (Fig.6 A). Furthermore, exposure to UV-C for 15 min/day tempted mild disruption of the cell junctions of the most superficial layer of the corneal epithelium (Fig.5B) and thickening of the wall (Fig.6B).

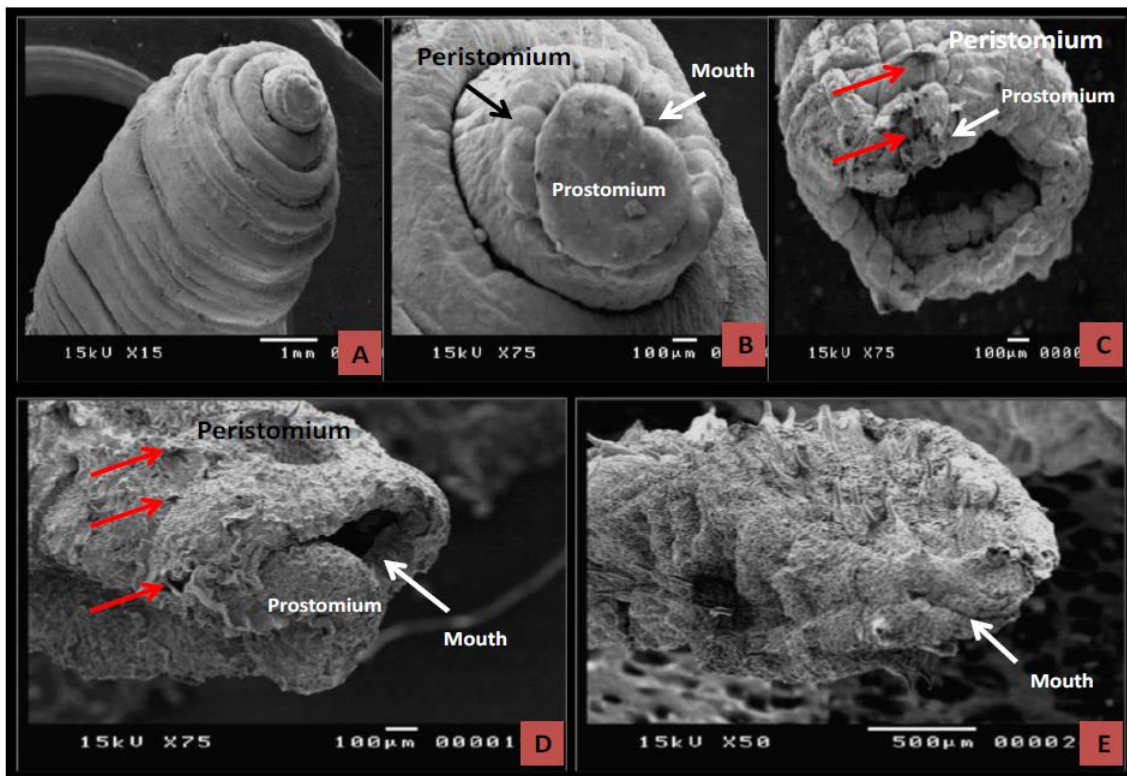
On the other hand, Sloughed tissues with an accumulation of crusts between junctions of the compound eye were observed after exposure to UV-C for 30 min/day (Fig.5C). The beginning of the disappearance of triangular scale-setae with hyperplasia of the epithelial layer also appeared (Fig.6C). However, exposure to UV-C for 60 min/day caused an increase in secretions with crusts between junctions of compound eyes (Fig.5D) and complete absence of triangular scale-setae with thickening of the epidermis

(Fig.6D).

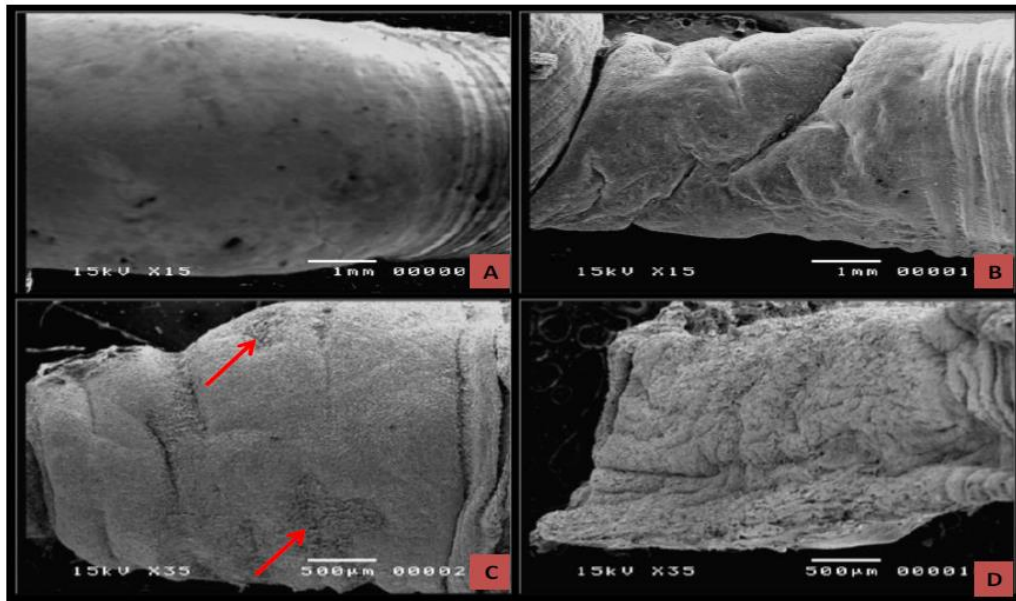
#### Mortality of Earthworms and Isopods Exposed to UV-C:

The earthworms mortality; For Group (1) without exposure to UV-C no mortality; Group (2) was about 16.67% (Fig.7A), Group (3) was about 30% (Fig.7B) and Group (4) was about 33.33% (Fig.7C) after exposure to UV-C for 15 min, 30 min and 60 min; respectively. Concerning isopods; For Group (1) without exposure to UV-C no mortality. For Group (2) was about ~22.22% (Fig.7D), Group (3) was about 28.89% (Fig.7E) and Group (4) was about 31.11% (Fig.7F); exposure to UV-C for 15 min, 30 min and 60 min led to mortality; respectively.

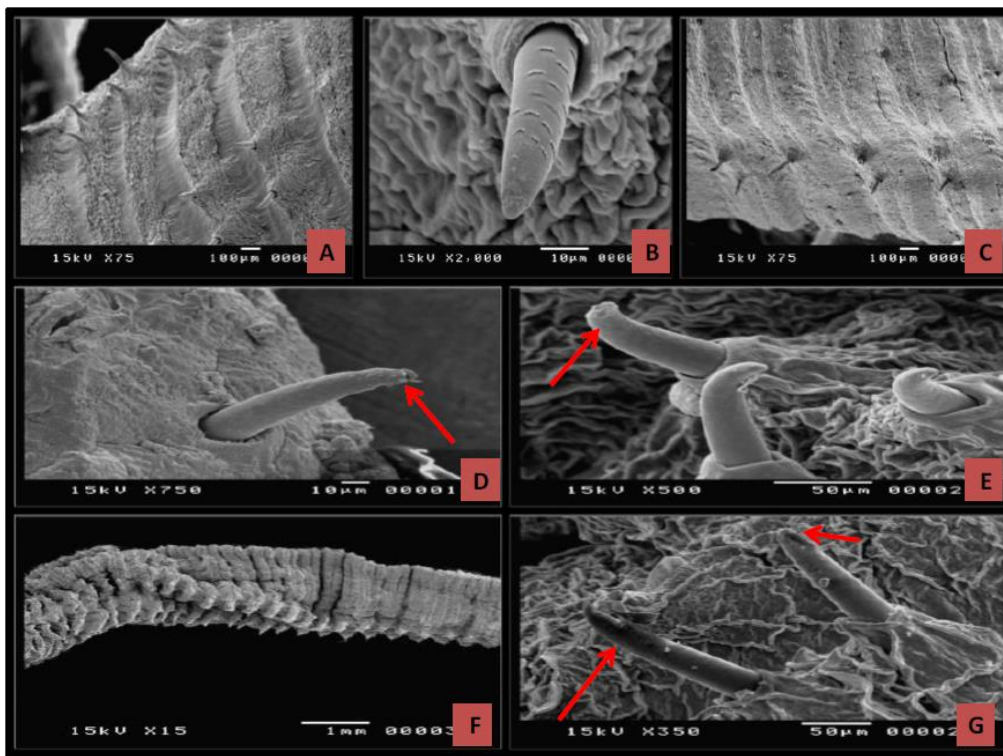
In addition, the mortality for earthworms exposed to UV-C; on the first day was about 0 %, on the second day was about ~43.3% and on the third day ~64.7%. The mortality isopods exposed to UV-C was; on the first day was 0 %, on the second day was about ~53.5%; and on the third day 72.4%. Finally, all earthworms and isopods died within 72h after UV-C exposure. This means that the UV-C dosage exposure and mortality rate of earthworms and isopod showed significant values at ( $P < 0.01$ ) and are highly significant at ( $P < 0.0001$ ).



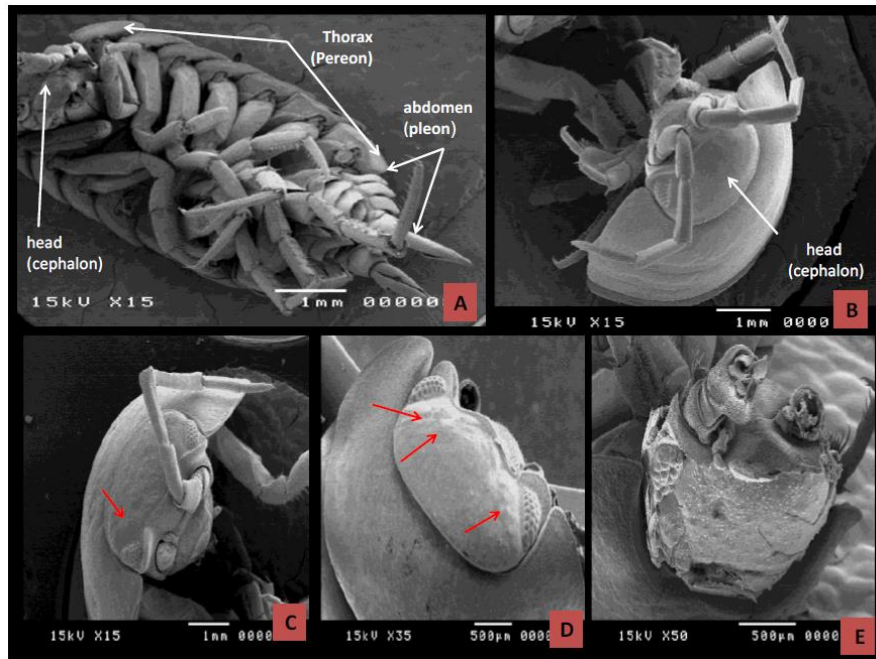
**Fig. 1:** Scanning electron photomicrograph of the anterior part of earthworm *Lumbricus castaneus* (A) without UV-C, (B) enclosed of showing prostomium, peristomium and mouth, (A), (C) Exposure to UV-C for 15 min/day showing dryness of the skin with formation of ulcer (arrows), (D) Exposure to UV-C for 30 min/day showing disappearance of the cell junctions of the most superficial layer (arrows) and (E) Exposure to UV-C for 60 min/day showing severe ulcer and complete closure of the mouth opening (arrows).



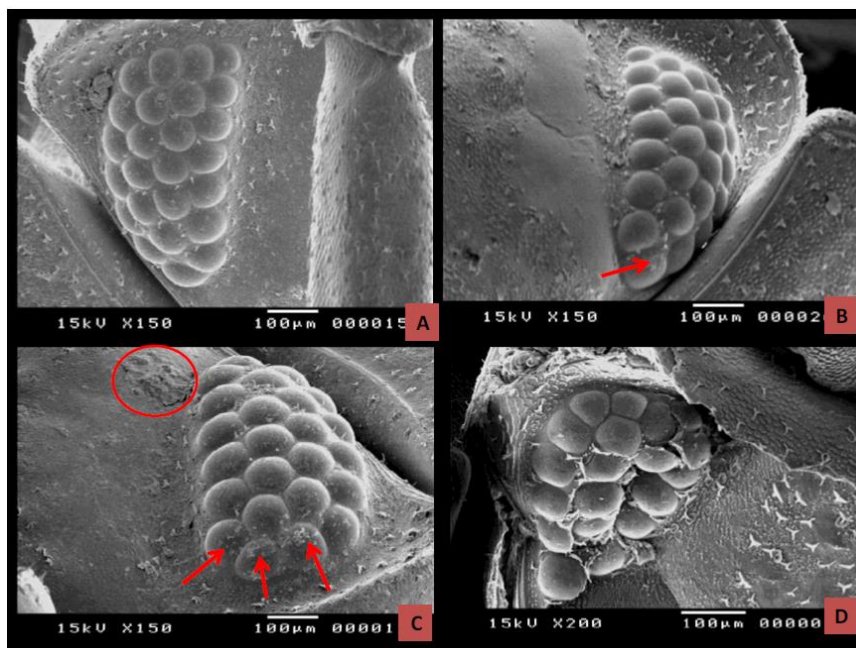
**Fig. 2:** Scanning electron photomicrograph of clitellum of earthworm *Lumbricus castaneus* (A) without UV radiation, (B) Exposure to UV-C for 15 min/day showing irregular groves and tunnels (C) Exposure to UV-C for 30 min/day showing dryness and erosions (arrows), and (D) Exposure to UV-C for 60 min/day showing necrosis and desquamation of epidermis.



**Fig. 3:** Scanning electron photomicrograph of earthworm *Lumbricus castaneus* (A), (B) body segment and setae without UV radiation, (C), (D) body segment and setae exposed to UV-C for 15 min/day showing a dryness of the outer layer with mild damage of the setae ends (E) body segment and setae exposed to UV-C for 30 min/day showing broken ends of setae, (F) body segment and setae after 60 min/day showing deep and separated transverse grooves and (G) body segment and setae exposed to UV-C for 60 min/day, showing crusts formation on the body with damaged setae.

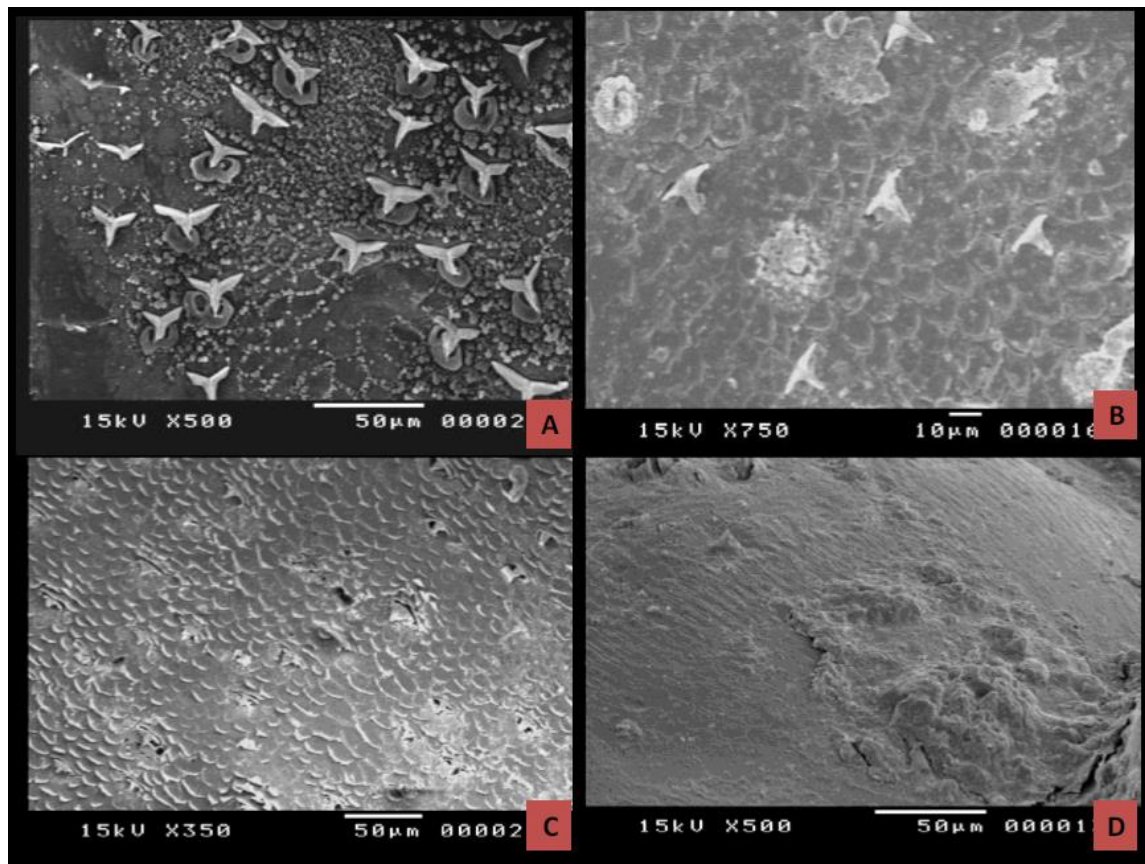


**Fig. 4:** Scanning electron photomicrograph of isopod *Porcellio laevis*, (A) Ventral view of the whole body without UV radiation, (B) anterior part of (A), (C) anterior part exposed to UV-C for 15 min/day showing mild swelling (arrow), (D) anterior part exposed to UV-C for 30 min/day showing marked multiple swellings (arrows) and (E) anterior part exposed to UV-C for 60 min/day showing severe damage in head accompanying with complete loss of one eye.

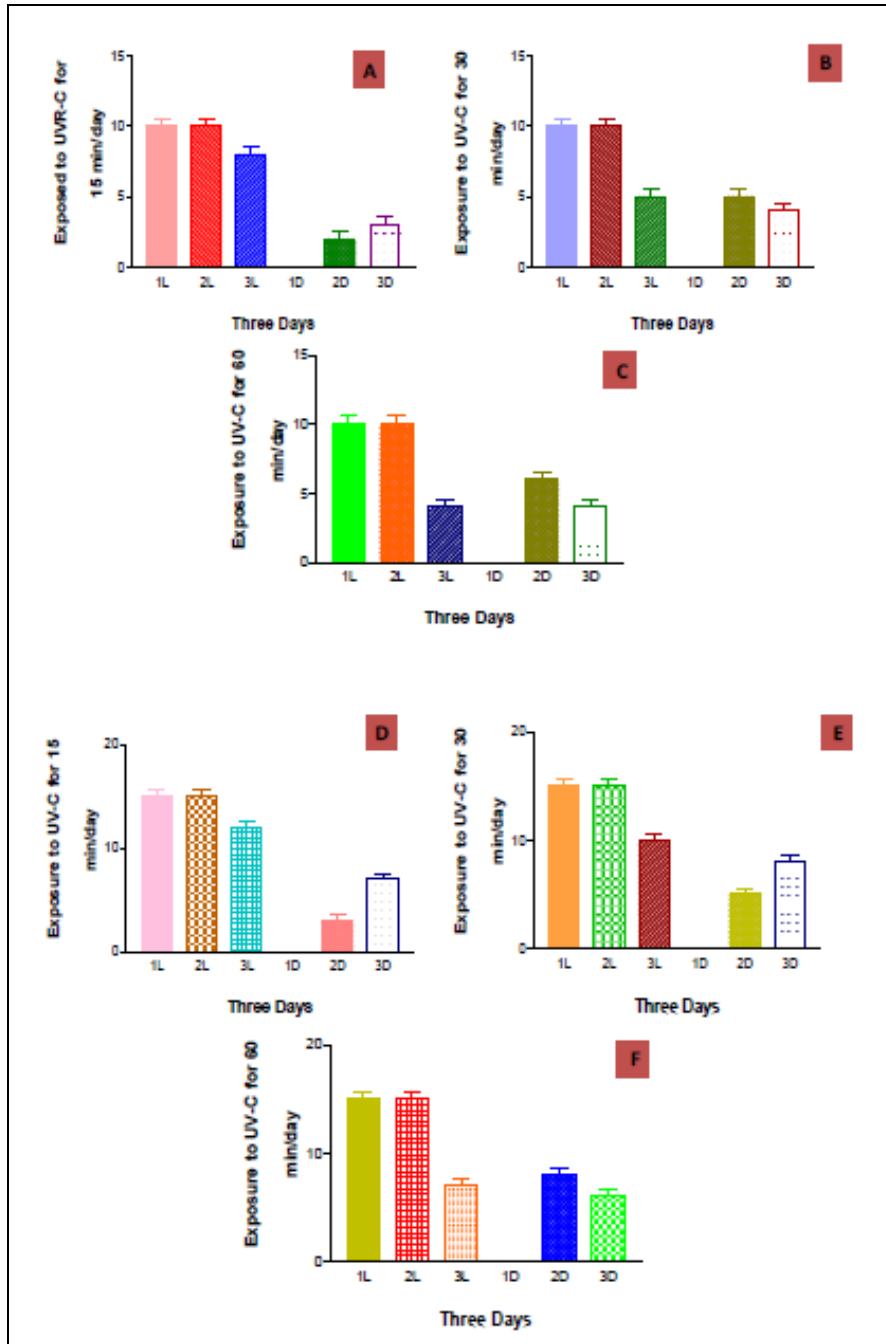


**Fig. 5:** Scanning electron photomicrograph of compound eyes of isopod *Porcellio laevis* (A) without UV radiation, (B) Exposure to UV-C for 15 min/day showing disruption of the cell junctions of the most superficial layer of corneal epithelium, (C) Exposure to UV-C for 30 min/day showing sloughed tissue (circle) with an accumulation of crusts between junctions of compound eye (arrows), and (D) Exposure to UV-C for 60 min/day showing an increasing amount of secretions with crusts between junctions of the compound eye.





**Fig. 6:** Scanning electron photomicrograph of the body surface of isopod *Porcellio laevis* surround eyes (A) without UV radiation, (B) Exposure to UV-C for 15 min/day showing thickening of the wall (C) Exposure to UV-C for 30 min/day showing the beginning of the disappearance of triangular scale-setae with hyperplasia of the epithelial layer and (D) Exposure to UV-C for 60 min/day showing complete absence of triangular scale-setae with thickening of the epidermis.



**Fig. 7:** Mortality of earthworms after UV-C exposure (A) earthworms, *Lumbricus castaneus* shows significant mortality after exposure to UV-C for 15 min /day (One-way ANOVA,  $P < 0.1$ ), (B) earthworms, *Lumbricus castaneus* shows significant mortality after exposure to UV-C for 30 min /day (One-way ANOVA,  $*P < 0.0001$ ), (C) earthworms, *Lumbricus castaneus* shows significant mortality after exposure to UV-C for 60 min /day (One-way ANOVA,  $*P < 0.0001$ ), (D) isopods, *Porcellio laevis* shows significant mortality after exposure to UV-C for 15 min /day (One-way ANOVA,  $P < 0.1$ ), (E) isopods, *Porcellio laevis* shows significant mortality after exposure to UV-C for 30 min /day (One-way ANOVA,  $*P < 0.0001$ ), and (F) isopods, *Porcellio laevis* shows significant mortality after exposure to UV-C for 60 min /day (One-way ANOVA,  $*P < 0.0001$ ).

1L (1<sup>st</sup>-day life), 2L (2<sup>nd</sup>-day life), 3L (3<sup>rd</sup>-day life), 1D (1<sup>st</sup> day dead), 2D (2<sup>nd</sup> day dead) and 3D (3<sup>rd</sup> day dead).

## DISCUSSION

In the present investigation, we revealed that UV-C radiation caused damage to earthworms and isopods. The earthworm was chosen as a human skin model by AbdEllahet *al* (2019) and for the estimation of the phototoxic effects of UV radiation because of the presence of various triene and tetraene sterols in its skin like that of humans (Albro, et al.,1997 and Misra, et al., 2005). So, the influence of UV-C radiation on earthworms may be compared to that of the human in a certain degree.

The present study showed that the group exposed to UV-C for 60 min/day caused more severe degeneration of integument or skin cells compared to those exposed to UV-C for 15 min/day and 30 min/day (for three days). Severe ulcer, complete closure of the mouth opening, necrosis and desquamation of epidermis, deep and separated transverse grooves and crusts formation on the body with damaged setae were noticed in the former group. Our findings agreed with Misra *et al*, (2005).

The skin is the main organ of respiration and circulation in earthworms. Subsequently, we expected that the cause of the mortality of the earthworms was their skin damage that led to suffocation. This result agreed with a previous study (Chuang *et al*,2006).

The UV radiation acuity in sunlight is due to the exhaustion of the stratospheric ozone layer (Kolstad, 1998). Many authors explained that ultraviolet radiation has harmful effects on many organisms (Ballare et al.,2011 and Bornman et al.,2015, Chuang *et al*,2006, Herndon *et al*, 2018, Marshall *et al*,1996). Ordinary exposure to the sun especially facial skin promotes the formation of wrinkles. Where the UV rays cause the skin to harden and lose elasticity (Maeda, 2018).

In the control group, the morphology of the isopoda and its eye agreed with the previously described reports (Chamberlain *et al*,1986). SEM examination of the control group revealed that the structure was usually about 20 mm long and the isopod body was smooth and divided into three distinct regions: head (cephalon), thorax(pereon), and abdomen (pleon). Regarding the group exposed to UV-C for 15 min/day, changes in disruption of the cell junctions of the most superficial layer of corneal epithelium, thickening of the wall were observed.

Exposure to UV-C for 30 min/day caused a sloughed tissue with an accumulation of crusts between junctions of the compound eye, beginning of the disappearance of triangular scale-setae with hyperplasia of the epithelial layer. Furthermore, an increase in secretions amount with crusts between junctions of compound eyes and complete absence of triangular scale-setae with thickening of the epidermis were noticed in the group exposed to UV-C for 60 min/day.

The aforementioned findings may be elucidated according to other reported studies (Vargas *et al* .,2010, Gouveia et al., 2005, Godley *et al*., 2005, Meyer-Rochow and Tiang (1984). Vargas et al (2010) supposed that UV-A and UV-B altered specific oxidative parameters; however, the cell damage was more evident in the eyestalk of the crab *Neohelice granulata*. In contrast, Gouveia *et al*,(2005) found that pigments in crustaceans (crab *Chasmagnathus granulata*), could decrease damage caused by UV radiation in the cells. Godley *et al*. (2005) recommended the photoreceptor damage, caused by UV radiation based on the presence of free radicals. While Meyer-Rochow and Tiang (1984) revealed that the changes affecting the rhabdom were probably linked to losses in visual sensitivity of the crayfish.

The mortality rate in this study was about 43.3%earthworms and 53.5% isopods in 24 hrs. The mortality in 48 hrs was about 64.7% of earthworms and 72.4% of isopods. All earthworms and isopods died within 72hrs after UV-C exposure. However, Misra *et*

*al.*, (2005) recorded 10% mortality of the earthworms after 3 hours while Chuang et al (2006) revealed the percentage mortality was about 60% in 48 hr. Chelladurai et al (2020) When exposing earthworms to higher doses of UV-C, revealed that tissue inflammation is accompanied by death. UV-C, in particular, causes damage to skin layers while also mediating chloragogen and epithelial outgrowth in intestinal tissues.

In the present study, For the first time, scanning electron microscope analysis was recognized to detect the impact of UV-C radiation on earthworms and isopods, which suffered from great damage due to exposure to UV-C. In this work, we preferred the scanning electron microscope (SEM), which is one of the most powerful instruments available for study and review of the morphology of the microstructure and gives high quality and efficacy of details for the morphological structure. Further studies would be directed toward histological investigating mechanisms underlying this effect and how to protect them from damage.

#### **Conclusion:**

To the best of our knowledge, it is the first study that investigates the effect of UV-C radiation on both earthworms (as a model of human skin) and isopods by SEM.

SEM examination for earthworms exposed to UV-C radiation showed dryness of the skin with formation of an ulcer, necrosis and desquamation of epidermis. In addition, isopods showed mild swelling, disruption of the cell junctions, thickening of the wall, severe damage in head accompanying with complete loss of one eye, increasing amount of secretions with crusts between junctions of compound eye and complete absence of triangular scale-setae with thickening of the epidermis.

This study determines the influence of UV-C radiation that can be considered a risk factor for melanoma and cataracts. This study was designed for the assessment of health implications and planning of safety measures for human beings that will help the researcher to uncover the critical area of excessive exposure to UV carries profound health risks that were not able to explore. Thus, a new theory on completely avoiding sunlight is not healthy, but there are ways to help ensure you don't get too much sun.

#### **Conflict of interest:**

All authors declare that they have no conflict of interest.

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