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The Effect of UV-A and Various Visible Light Wavelengths Radiations on Expression Level of *Escherichia coli* Oxidative Enzymes in Seawater

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ABSTRACT

Background: Light and photosensitizers affectthe survival of bacteria in natural environments. Also light and photosensitizers are used for disinfection of materials such as blood, blood products, and water.
Objectives: The present study was aimed toinvestigate the effect of different wavelengths of visible light and UV-A on the synthesis of some oxidative stress enzymes of *Escherichia coli* (*E. coli*) in seawater.
Materials and Methods: Seawater were filtered by using Whatmann No:1 filter paper, followed by sterilization in the autoclave. The *E. coli* W3110 strain was grown at 37 ^{O}C, centrifuged, and transferred in seawater, then methylene blue was added to the seawater samples, with the exception of control samples. The seawater samples were incubated with white, blue, green, red, and UV-A light sources. Cell extracts were prepared by sonication, and then catalase, superoxide dismutase (SOD), glutathion peroxidase(GP),and glucose-6-phosphate dehydrogenase(G-6-PD) activities were measured.
Results: It was found that in all studied wavelengths with or without Methylene Blue (MB), the level of all studied enzymes decreased remarkably when compared to dark controls. It was observed that the synthesis level of SOD, glutathione peroxidase GP, and glucose 6 phosphate dehydrogenase G-6-PD in *E. coli* decreased significantly in red light with respect to white, blue, and green light in seawater, to which methylene blue was added. In *E. coli* the decrease was 13% of G-6-PD expression, 10% of GP expression, and 17% of SOD expression in red light with MB after 16-hour incubation in seawater; however, these enzymes decreased to 45%, 84%,and 71% in white light, 33%, 47%, and 54% in blue light, 53%, 53%,and 64% in green light at the same incubation hours, respectively. Also, the enzyme acitivity in red light without MB did not show a significant difference when compared to other light sources.
Conclusions: It was shown in the present study that red light among visible light sources has a crucial effect in decreasing the oxidative stress enzymes in seawater containing MB.

Keywords: Red light; UV-A; Methylene Blue; *Eschericia coli*; Seawater; Photo-oxidation

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Implication for health policy/practice/research/medical education:
This study contributed to the understanding of the effect of red light at areas used in photo-oxidation such as in public health and medicine. Also this study has shown the survival strategies of *Escherichia coli* under photo-oxidative stress in natural environments.

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1. Background

Solar radiation is one of the most important factors affecting the survival of enteric bacteria in aquatic environments (1-3). Solar radiation that reaches the earth consists oftwo types: visible (400-700 nm) and UV (285-400 nm). Visible light includes blue (400-500 nm), green (500-600 nm), yellow (565-590), and red (600-700 nm) wavelengths, and UV light includes UV-A, UV-B, and UV-C wavelengths. Previous studies have shown that the hazardous effect of light on bacteria is further increased by light-sensitive molecules known as photosensitizers (4-7). While porphyrin, riboflavin derivatives, and hemin are termed as endogenous photosensitizers; humic acid, photosynthetic pigments, and various stains (such as Methylene blue-MB) are termed as exogenous photosensitizers (8-13). Light produces reactive oxygen species (OH-, $O2^{-}$, $^{1}O2$, H2O2) from photosensitizers, and these molecules causes serious damage to all cellular molecules.

Reactive oxygen radicals are comprised of unpaired electrons. These radicals are unstable, react rapidly with other molecules, and need to acquire electrons in order to become chemically stable. When reactive oxygen radicals attack a molecule, they oxidemolecules by stealing its electron, and turn into new molecules as free radicals (14). A series of chain reactions are triggered in this way during the death of living cells. These radicals cause serious damage to proteins, DNA, fatty acids in membranes, and organelles (15-18).

Bacteria resists the photooxidative stress with enzymatic or nonenzymatic mechanisms. Enzymatic mechanisms contain enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GP), and G-6-P dehydrogenase (G-6-PD); whereas nonenzymatic mechanisms contain glutathione and riboflavin(19, 20). In addition, protective mechanisms such as changing membrane permeability with porin proteins are also important (7). Numerous studies are available in the literature which focus on the effects of light on bacteria (21, 22), however, there are very few articles aboutthe effects on enzyme production resulting fromdifferent wavelengths of visible light.

It was shown in a previous study (23) that when Escherichia coli in seawater containing MB was exposed to UV-A and various wavelengths of visible light (red, white, blue, green) as a photosensitizer, cell population decreased by 99 % according to (t99)at 6.8, 10.2, 19, 21.3 and 24 hours, respectively. The effects of red light alone in seawater without MB were also explored in the same study, it was reported that cell population decreased by 99 % in the effects of red, UVA, white, blue and, green light at 24.4, 24.6, 56.6, 59.5 and 66.0 hours, respectively. These findings revealed that red light was the most effective among visible wavelengths, and it was as effective as UV-A. Accordingly, it was concluded that red light without a photosensitizer was considerably more effective than other visible wavelengths on the survival of bacteria (23), as in samples containing MB. Why is red light effective on the life of bacteria? (4, 23-25).Well, till now there is not enough literature on the reason of effectiveness of red light on bacteria's survival, where this study would hopefully find an answer.

2.Objectives

The aim of this study was to investigate the effect of various wavelengths of the light spectrum on the expression level of oxidative stress enzymes of *E. coli* in sea water.

3. Materials and Methods

3.1. Bacteria and the Environment Used in the Experiment

Seawater was obtained from the Samsun-Turkey coastline and its physical particles were eliminated by using Whatmann No 1 (Whatmann-USA) filter paper, followed by sterilization in the autoclave (Nüve-Turkey). The *E. coli* W3110 strain was cultured in a 150 rpm agitator incubator (GFL-Germany) using 50 ml nutrient broth (Merck-Germany) at 24 h. From these cultures, a 15 mL bacteria sample was centrifuged, rinsed, dispersed by seawater, and transferred in 100 mL seawater in 250 mL beakers (final concentration of $1.5x10^{9}$ CFU/ml). Subsequently, MB($1,5.10^{-6}$ M final concentration) (Merck-Germany) was added to the seawater samples, with the exception of control samples.

Radical production (photo-oxidation) was achieved by exposure of MB to light. Some seawater microcosms were incubated without irradiation as a negative control (dark control), and some were irradiated but without MB as a positive control (light control). The toxicity of MB in bacteria was controlled with the dark control containing MB. The top portions of the beakers were wrapped with a cling film in order to prevent contamination. The seawater samples were incubated for 9 h withUV-A radiation, and for 16 h with MB or 24 h without MB under different visible light wavelengths at 24 ^{0}C. . We used different periods of time becasue of the variance of survival times of *E. coli* under different light sources (23).

3.2. Light Sources

In the present study, white (400-700 nm), blue (400-500 nm), green (500-570 nm), red (600-700 nm), and UV-A light sources were used. For visible light sources, eight Osram L 18W/66 fluorescent lamps were used, whereas four Osram Eversun L 40W/79K fluorescent lamps were used for UV-A (Osram-Germany).

3.3. Expression Level of Antioxidant Enzymes

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15 mL seawater samples were obtained, centrifuged at 8000 xg and rinsed twice with potassium phosphate buffer. These samples were then mixed by adding potassium phosphate buffer and 0.1mM EDTA on the pellet obtained. Cell extracts were prepared by sonication (Fisher-Sonic Model 300). Further cellular debris werere moved by 15-min centrifugation at 15,000xgat 4 ^{0}C. The extracts were maintained at temperatures below -25°C for use in supernatant analyses. For the analyses, protein levels were determined by using bovine serum albumin as the standard (26).

Catalase activity was measured in terms of hydrogen peroxide decomposition, which was followed directly by a decrease in absorbance at 240 nm spectrophotometrically (27). The cell extracts were analysed spectrophotometrically using the method of Flohe and Otting (28) for the SOD activity, with the Leopold and Wolfgang method (29) for the GPenzyme activity, and with Hylemon and Phibbs method (30) for the G-6-PDactivity. All enzyme activities were measured as U/mg protein.

3.4. Data Analysis and Statistics

Each experiment was performed at least in triplicate. Enzyme activities were expressed as a percentage. Expression level of enzymes at 0-hour is expressed as 100%. Differences between treatments and the time dependent change were analyzed using the Student'st-test ($P < 0.05$).

4. Results

Methylene blue (MB) was used as a photosensitizer in order to analyze the changes in oxidative stress enzymes of *E. coli* under the effect of photo-oxidation in seawater. The radicals consist of MB when exposed to light. The activities of catalase, SOD, GP, and G-6-PD enzymes of *E. coli* subjected to different wavelengths were measured. The enzyme synthesis levels are presented in *Figure 1* and *Table 1*. Level of enzymes at 0-hour is expressed as 100%.

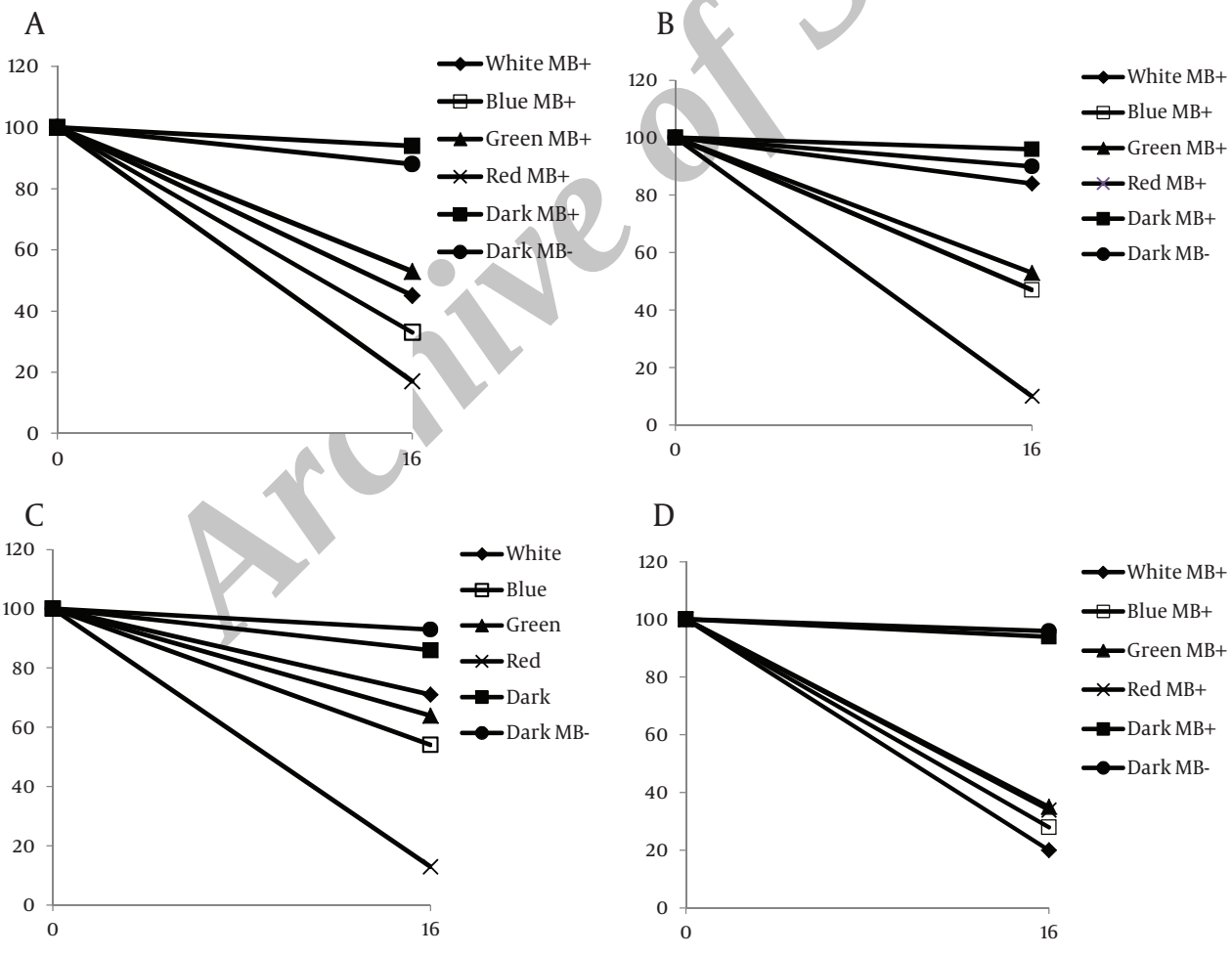

Figure 1. The Percentage of Enzyme Activities of *E. coli* Under the Effect of Different Wavelength Light Sources in Seawater Containing MB.

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Table 1. The Percentage of Enzyme Expression Level of *E. coli* Under the Effect of Different Wavelength Light Sources in Seawater.

		Enzyme Levels, %							
		SOD		CAT		GP		G-6-P-D	
Light Sources	0- h (%)	16 h MB (+)	24 h MB (-)	16 h MB (+)	24 h MB (-)	16 h MB (+)	24 h MB (-)	16 h MB (+)	24 h MB (-)
White	100	45	32	20	27	84	44	71	69
Blue	100	33	36	28	31	47	67	54	15
Green	100	53	62	35	23	53	55	64	64
Red	100	17	53	34	23	10	44	13	53
Dark Control	100	94	92	94	92	94	88	86	92
	0- h (%)	9 h MB (+)	9 hMB (-)	9 hMB (+)	9 hMB (-)	9 hMB (+)	9 hMB (-)	9 h MB (+)	9 hMB (-)
UV-A	100	44	58	12	16	32	39	40	47
Dark Control	100	96	92	95	89	96	89	86	86

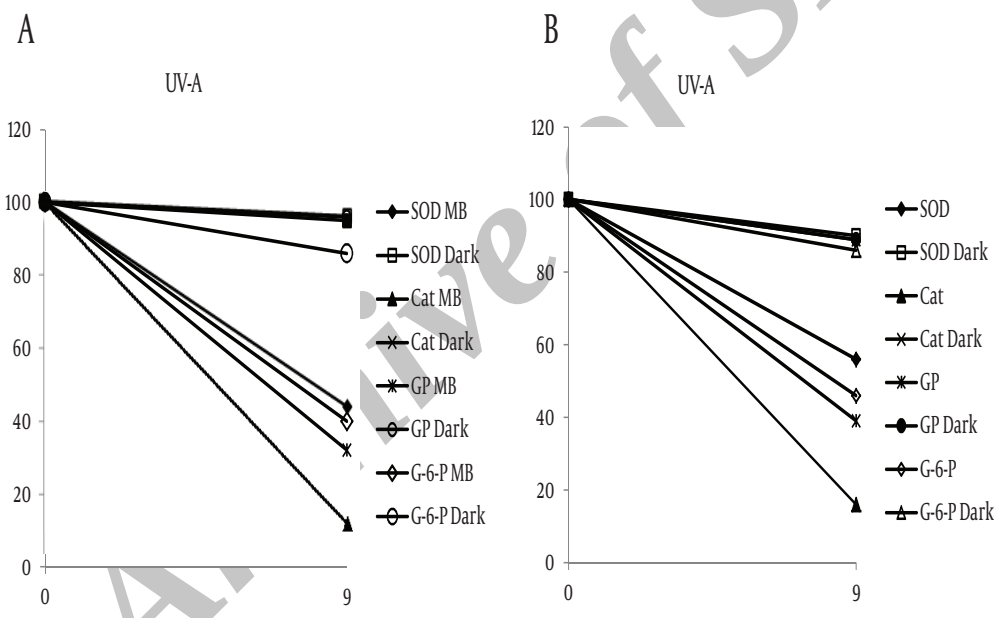

Figure 2. The Percentage of Enzyme Expression Level of *E. coli* Under the Effect of UV-A Light Source in Seawater.

It can be seen that when MB was added, the incubation time was16 h at visible light sources *(Figure 1)* and 9 h at UV-A (*Figure 2 A*). UV-A killed a lot of bacteria at visible light effective time (16 h). Therefore, we tried different periods of time. When *E. coli* was exposed to light sources of different wavelengths with MB in the seawater, all enzymes showed reduced synthesis level. The SOD, catalase, and GP activities were reduced to 94% in the dark control samples, and the G-6-PDexpression level was reduced to 86%. A comparison of the enzyme expression level in dark controls and the illuminated samples indicated that the synthesis of all enzymes had been reduced ($P < 0.05$).

Among the light sources, the red wavelength was found to be the most effective (*Figure 1* and *Table 1*) ($P < 0.05$). While SOD, GP, and G-6-PD expression level showed significant reduction in red light, catalase level showed reduction at a rate similar to other visible light sources ($P > 0.05$). It was seen that SOD expression level decreased to 17% in red light, 33% in blue light, 45% in white light, and 53% in green light. Similarly, GP synthesis level decreased to 10% in red light, 47% in blue light, 44% in white light, and 53% in green light; and G-6-PD expression level was similarly reduced to 13% in red light, 54% in blue light, 71% in white light, and 64% in green light.

When *E. coli* was incubated under UV-A light for 9 h, it decreased SOD expression level to 44%, catalase expres-

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sion level to 12%, GP expression level to 32%, and G-6-PD expression level to 40% (*Figure 2 A*). The photo-oxidative stress formed by MB led to a decrease in *E. coli* enzyme expression levels. It was observed that among visible wavelengths, red light in particular had a significant effect on SOD, GP, and G-6-PD . The effect on catalase enzyme expression level was almost similar under all light sources (P >0.05). The catalase enzyme sytnhesis level under photo-oxidation was more affected according to other studied enzymes.

E. coli were observed for 24 hours in white light, blue light, green light, red light, and in 9-hour incubations under UV-A in seawater without MB according to survival time (23). It was seen that in all the light sources studied in seawater, the SOD, catalase, GP, and G-6-PD activities of *E. coli* were significantly reduced when compared to dark controls (*Figure 3* and *Table 1*) (P < 0.05). Also the effectiveness of red light could not be seen when photosensitizers (MB) were not present (*P* > 0.05). The most note worthy result was obtained in the activitiy of the G-6-PD enzyme under blue light, where this enzyme decreased to 15%, whereas it decreasedto 69% under white light, 64% under green light, and 53% under red light. Catalase synthesis level was found to be the most affected under the effect of light alone as well as with MB added (*Figure 3* and *Table 1*).

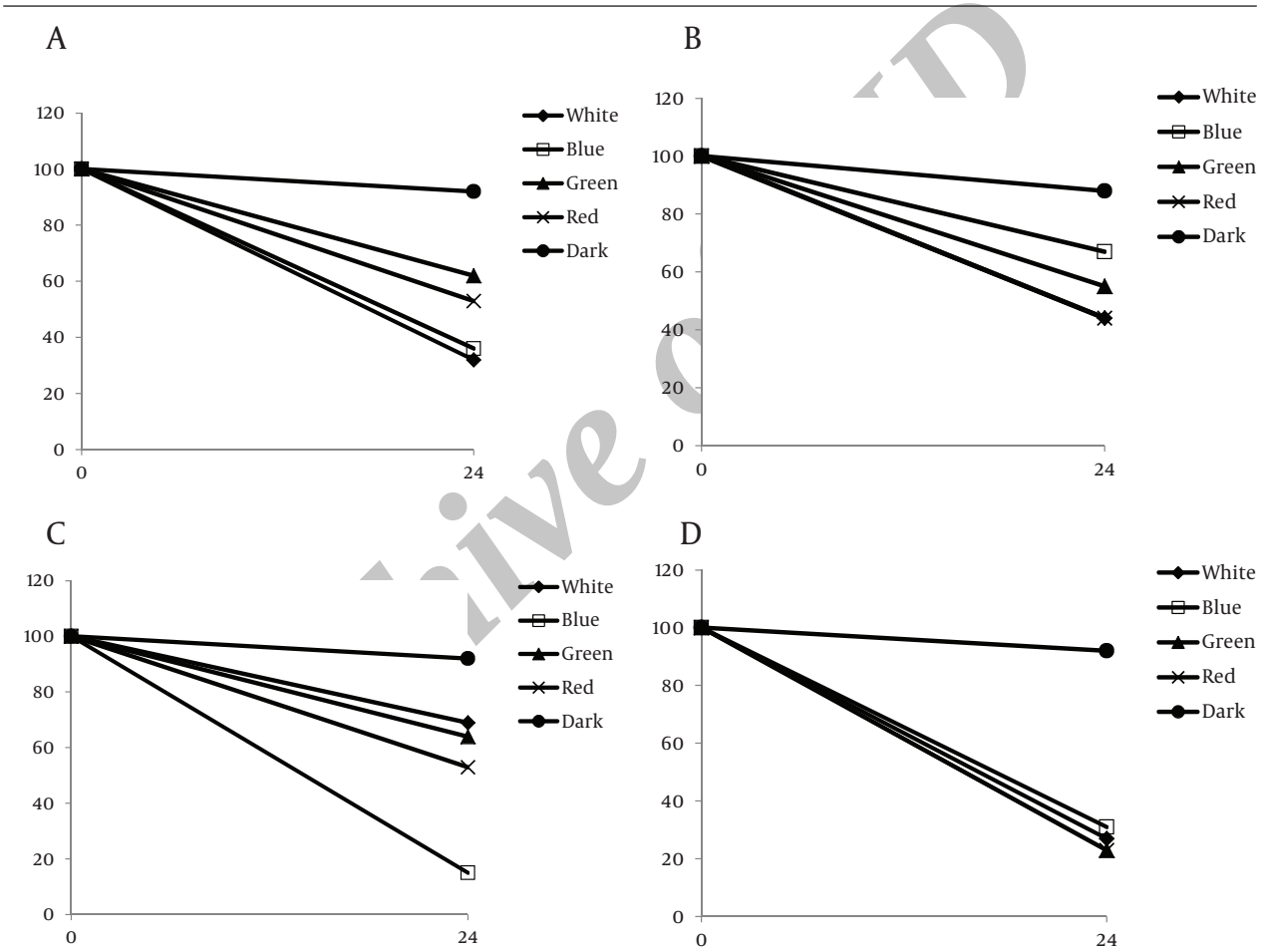

Figure 3. The Percentage of Enzyme Expression Level of *E. coli* Under the Effect of DifferentWavelength Light Sources in Seawater Without MB.

5. Discussion

Oxidative stress occurs with reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radicals that damage proteins, DNA, lipid, and the cell membrane. Light sources have considerably stronger effects with reactive oxygen radicals which occur in combined form with natural photosensitizers, such as humic acid orprotoporfirin;(31, 32)or with artificial photosensitizers, such as methylene blue, toluidine blue, or rose bengal (33, 34). The radicals consist of MB when exposed to light. The wavelengths of light spectrum have different effects on bacteria in aquatic environments (23, 24). Bacteria are known to have protective mechanisms against stress, which can be divided into two types; enzymatic and non-enzymatic. They are protected against stress and

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can prolong their life by means of enzymatic protection mechanisms, such asSOD, catalase, GP, and G-6-PD .

MB has been used as a photosensitizer in numerous studies (33-35). In the present study, it was observed that SOD, GP, and G-6-PD enzyme activities were greatly reduced under red light in seawater containing MB. Maximum absorbance of MB was displayed at a wavelength of 664 nm (13). When treated with red light, MB creates singlet oxygen radicals (13, 36). Thus, its maximal absorbance at red wavelength indicates that it will cause more radicals to form, which plays a major role in bacteria death. Bacteria increase the synthesis of various enzymes for protection against oxidative stress. However, it should be noted that there was a decrease in the four enzymes of *E. coli* when studied in seawater, which leads toa poor nutritional environment. The reduction of enzyme synthesis level in red light in the presence of MB has been attributed to the fact that the maximal absorbance wavelength of this stain is 664 nm. In seawater without MB, red light has similar effects on the level of enzyme synthesis as other light sources.

The effects of UV-A remain independent of the presence of MB. UV-A is known to be more effective on bacteria as compared to visible light wavelengths (37). So, enzyme synthesis should be reduced more in UV-A than in visible wavelengths. This is because enzyme synthesis is greatly reduced in visible wavelengths.

Enzyme synthesis levels have been shown to decrease with the effects of light independently without MB. Troussellier *et al.* (38) studied *E. coli* and *S. typhimurium* in seawater, and concluded that SOD and catalase enzyme activities did not vary significantly in samples subjected to white light as compared to dark control samples, and a decrease was observed over time. Similarly in the present study, it was found that enzyme synthesis levels decreased in all light sources as compared to dark control samples (with or without MB).

As an interesting finding, G-6-PDenzyme synthesis levelwas significantly reduced more under blue light (with MB) than under other light sources (with MB). The G-6-PD enzyme is the first enzyme of the pentose phosphate pathway. As a reducing agent, this is the most important enzyme usedto obtain NADPH2 which plays a role in oxidizing reduced glutathion. Thus, G-6-PD is known to play a very important role in protecting against oxidative stress via glutathion (19, 39).

Although antioxidant enzymes play an important role in the defense mechanisms of bacteria, these enzymes are rather sensitive to the inactivation with reactive oxygen radicals (36, 40, 41). It has been determined that the loss of enzyme activities by radicals occurs as a result of fragmentation and agregation of peptides by the formation of carbonyl groups. Studies aiming to determine the target areas in enzymes affected by radicals have shown that 48% of histidine residues are lost in SOD, and 32% in catalase (36, 42, 43). Thus, it can be concluded that the singlet oxygen formed as a result of the treatment of MB with light plays a crucialrole in the inactivation of enzymes. Furthermore, catalase enzyme expression level was found to be decreased by a similar amount in all sources of light studied, where this enzyme is rather sensitive to radicals.

İdil *et al.* (23) showed that red light was more effective than other light wavelength in the formation of viable but non culturable state (VBNC). The mechanism of VBNC state is however not clear yet. Several studies have suggested that the EnvZ osmosensor could play a key role in the entry to VBNC state (44). As the dormancy that might occur due to light and oxidative stress is important for public health, and as light is used for photodynamic therapy and water disinfection such as solar disinfection, it is also essential to understand the defense mechanisms of bacteria and the molecular mechanisms of the damage. It would also beuseful to explain the physical and molecular mechanisms of the effects of red light on bacteria.

To conclude, red light is mostimportant in the treatment of MB with light, as enzyme synthesis is considerably most affected under this condition. It was found that the enzyme synthesis was not enough to protect the bacteria under photo-oxidative stress in seawater; where in fact, enzyme synthesis levels were seen to decrease significantly. The present study offers an insight into bacterial life and enzyme synthesis levels under photo-oxidative stress induced by different wavelengths. A complete understanding of the molecular mechanisms of the damaging of cells during red light treatment is still required.

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Authors' Contribution

None Declared.

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