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Toxicity Effect of Cu^{2+} Contaminated Kitchen and Vegetable Wastes on Oxidative Stress Response of Black Soldier Fly Larvae, *Hermetia Illucens*

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Abstract

Environmental pollution sources including waste or metal accumulation, industrial and agricultural activities can be dangerous. Also, contaminated organic waste (COW) with metals especially, copper ions (Cu^{2+}), can cause toxicity to various ecosystem components, enhance the production of reactive oxygen species (ROS) and consequently cause oxidative stress. The biochemical effect of the COW was monitored by assessing the oxidative stress parameters (OSP) using hydrogen peroxides (H_2O_2), protein carbonyls (PC), lipid peroxides (LP), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), 2,2 diphenyl-1-picrylhydrazyl (DPPH), total antioxidant ability (TAA); β-esterase (β-EST); and total amount of protein (TAP) levels on the organic waste (kitchen and vegetable wastes); *Hermetia illucens* larvae; and larval excreta collected from 7-day post-treated kitchen and vegetables wastes with (1:10; g:mL) distilled water (DW) or 100 mg/mL Cu^{2+}. The OSP levels were significantly higher in the experimental samples from Cu^{2+} groups than in the control one. Besides that, the OSP levels of *H. illucens* larvae feed on vegetable waste was significantly higher than those feed on kitchen waste except for β-EST, PC, and TPA. The best, cheap and easy parameters of antioxidants to ensure the entomoremediation ability are total antioxidant capacity. Possible impacts of accumulated and Cu^{2+} contaminated organic waste on *H. illucens* larvae were discussed. Also, the ability of insects to produce more antioxidants than input or output sources was approved. The potential use of the OSP as a bioindicator method of the bioremediation ability of *H. illucens* was proposed.

Keywords: Black soldier fly; Bioremediation; Organic waste; Oxidative stress; Toxicology; Cu^{2+}.

1. Introduction

The effects of various chemical or environmental pollutants on the insects' physiology and biochemistry have been investigated in various studies [1-9]. Environmental pollution sources including waste accumulation, industrial and agricultural activities have a direct effect on the amounts of cupper elevation than normal levels. Excess of

copper ions (Cu^{2+}) can cause toxicity to various tissues [10], enhance the production of reactive oxygen species (ROS) and consequently cause oxidative stress in living organisms, such as insects [5, 11-14]. Yet, Cu2+ are crucial for cellular biochemistry and physiology, as it is used as a cofactor of various enzymes, and factors of cellular processes such as oxidative stress defense, ion homeostasis, cellular respiration, neural transmission, tissue maturation, or iron metabolism [15, 16]. The deficiency of Cu2+ deficiency leads to the loss of the functions of several enzymes, such as superoxide dismutase (SOD), cytochrome -c- oxidase, lysyl oxidase, and dopamine-b-hydroxylase [17]. The main function mechanism of Cu^{2+} in living organisms is linked with its redox chemistry [3, 5, 18].

Metals especially copper can occur in the organic waste [19]. Besides that, organic waste accumulation and contamination can accelerate the negative actions of climate change and increase harmful emissions because of open dumping and burning [20, 21]. Therefore, improper organic waste management may cause a deleterious effect on the ecosystem components [22] and act as a source of many diseases [23] as a result of emissions of polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs), greenhouse gases (CH_4, CO_2 and N_2O). The toxic emissions waste can lead to air pollution, global warming effect and exacerbated consequences associated with oxidative stress including changes in the macromolecules amount, elevation the levels of protein carbonyls (PC), lipid peroxides (LP) or DNA strand breaks [2, 5, 8, 14, 23-26]. Besides that, many previous studies showed an increase in the heavy metals, especially Cu, Cr, and Cd, concentrations in the organic manure and waste [27, 28].

Biological systems can cohabit with the deleterious effect of oxidative stress and free radicals' production through effective antioxidants mechanisms. There are two antioxidant defenses mechanisms: one is enzymatic, while the other is non-enzymatic [5, 29, 30]. The nonenzymatic includes β-carotene, α-tocopherol [14], and 2,2 diphenyl-1-picrylhydrazyl (DPPH) [31], can neutralize free radicals, as hydrogen peroxide (H_2O_2), and counteract the harmful effects of oxidative stress [29](. In addition to, SOD, catalase (CAT), glutathione-S-transferases (GSTs), glutathione peroxidase (GPx), β-esterase and ascorbate peroxidase (APOX) are antioxidant and detoxification enzymes that act as a critical defense line against ROS [8, 32-34]. So, the measurement of the oxidants, antioxidants, and damaged products levels can be done to quantify the oxidative stress [5, 8, 14]. Besides that, the total antioxidant capacity can be used as a single, easy, quick and cheap method to detect oxidative stress [35].

The use of insect biological processes to degrade environmental toxins, and heavy metal from ecosystem components especially soil, and water is called entomoremediation [36]. The *in situ* solutions development for remediating environmental toxins has attracted a lot of attention [36-38]. Collembolans, ants, flies, beetles and termites are considered as ecosystem engineers, entomoremediaition agents of toxins, nutrient cycling improvers, ecosystem health recoverer, fertilization agents and even toxicological risk assessment tools [36, 38-46]. The mechanism of insect detoxification ability may depend on metals storage inside the midgut epithelia of soil invertebrates, and extra metal excretion during moulting and renewable midgut epithelium [36]. Besides that, the generation of fungal/microbial/insectal-enzymes can increase the bioremediation ability of the waste stream and the production ability of high-value products, including industrial antioxidants enzymes [47]. Moreover, BSF larva (BSFL) has the ability to convert a broad range of contaminated organic wastes and side streams into larval biomass, so, it can serve as an alternative eco-friendly bioremediation tool [42]. Recently, biological research on BSFL has risen in conjunction with the growth of industrial mass manufacturing [48]; the determination of waste management effectiveness for different waste-stream diets [49]; the exploration of the BSFL production impacts on the environmental quality [50, 51]; and the determination of food or feed safety and security [49, 52, 53].

In the present study, we want to answer some questions; can we manage organic waste contaminated with metals using BSFL? What is the validity of using oxidative stress parameters as an indicator of bioremediation ability? Are BSFL having the ability to produce more antioxidants especially within polluted zones? Therefore, the main aim of the present work is the measurement of oxidative stress and detoxification levels in the various types of organic waste, BSFL, and insect excreta. So, the levels of H_2O_2, PC, LP, CAT, GPx, SOD, β-esterase, DPPH, total protein amount and total antioxidant capacity were measured in the Cu^{2+}-contaminated vegetable or kitchen waste, 5th instar *Hermetia illucens* homogenates and insect excreta.

2. Materials and Methods

2.1. Insect Treatment and Sample Preparation

From the Entomology Department, Faculty of Science, Cairo University, Egypt, the 5th instar larval of *H. illucens* was supplied from a colony reared under the rearing conditions (0:24 L:D; 34˚±2; 75% RH). The organic wastes (kitchen and vegetable) were provided routinely from the Giza governorate houses. Each 10 mL of Cu^{2+} (100 mg/mL) or 10 mL distilled water was mixed with 1gm organic waste. For each sub-group, different organic waste sources, 50 insects and their feces substances were collected, after 7 days post-application for further analysis and were stored at −20 °C until use. Each experiment was done in three replicates.

2.2. Reactive Oxygen Species and Oxidative Damage Concentration

By using a spectrophotometer, the H_2O_2 concentration of experimental samples determined by Junglee, *et al.* [54], 150 mg of each sample was directly homogenized with 1 mL of a solution containing 0.25 mL Trichloroacetic acid (TCA) (0.1% (w:v)), 0.5 mL KI (1 M) and 0.25 mL potassium phosphate buffer (10 mM, pH 7.0) at 4˚C for 10 min (one-step buffer: extraction and colorimetric reaction combined). The homogenated sample was centrifuged at 12,000 × *g* for 15 min at 4˚C. The absorbance was measured at 240 nm.

From Levine, *et al.* [55], we used the procedure for the protein carbonyls assay, with the below-described modifications. In 5 mL ice-cold phosphate buffer (60 mL of 50 mM phosphate buffer, 10 mL of 0.1% Triton X-100, 5 mL of 0.05 mM $CaCl_2$; then completed to 100 mL with distilled water after adjusting pH to 7.0 with 2M HCl or NaOH) samples were homogenized. The samples were centrifuged at 2000 ×*g* for 10 min at 4 °C after homogenization (mortar, 10 strokes/ 30 seconds), an 800 µl aliquot of the supernatant mixed with 200 µL of 10 mM 2, 4-dinitrophenyl hydrazine (DNPH) prepared in 2 M HCl. The samples were incubated for 30 minutes at room temperature, precipitated with 10% Tricholoroacetic acid (TCA), and left for 10 min at 4 °C. At 5000 ×*g* the samples were centrifuged for 7 min at 4 °C. The pellet was washed four times with an ethanol/ethyl acetate (1:1) mixture and redissolved in 1 mL of sodium phosphate buffer (60 mL of 150 mM phosphate buffer, 30 mL of 3% sodium dodecyl sulphate, adjusted to a final volume of 100 mL with distilled water after adjusting the pH to 6.8 with 2M HCl or NaOH). Finally, the absorbance was measured at 366 nm, and the rate of protein carbonyls concentration was expressed as OD/mg protein.

As Hermes-Lima, *et al.* [56] method, the lipid peroxides concentration was measured. The samples were homogenized in ice-cold methanol (1:5, w/v). At 4 °C, the samples were centrifuged at 2000 *g* for 10 min after homogenization (mortar, 10 strokes/30 seconds). For the assay, a 5 mL aliquot of the supernatant was used. The following components were consecutively added to the samples (200 µl of supernatant): 400 µl of 1 mM $FeSO_4$, 200 µL of 0.25 M H_2SO_4, and 200 µl of 1 mM xylenol orange. The absorbance was measured at 580 nm. Lipid peroxides concentration was expressed as mM cumene hydroperoxides/µg protein.

2.3. Determination of the Enzymatic and Non-Enzymatic Antioxidants Levels

After homogenization using mortar (30 strikes/1 min.), sample preparation of all assessed enzymatic antioxidants was done in 100 mM phosphate buffer saline (PBS) (pH= 7.0). Then centrifugation had done at 4000 ×*g* for 10 min at 4℃.

The measuring of SOD activity is based on the procedure described by Misra and Fridovich [57]. The reaction mixture was as follows: 0.4 mL of a sodium carbonate buffer (200 mM; pH 10.0), 35 µl of EDTA (10 mM), 87 µl of the supernatant and 0.5 mL of freshly prepared epinephrine (15 mM). The absorbance was measured at 480 nm. SOD activity was expressed as OD/µg protein/min.

The activity of catalase (CAT) was assessed in compliance with Aebi [58] method. The reaction mixture contained 0.9 mL of a potassium phosphate buffer (50 mM, pH 7.0), 60 µL of the supernatant and 40 µL of freshly prepared H_2O_2 (10 mM). The change in absorbance was measured at 240 nm for 1 min. The CAT activity was expressed as OD/µg protein/min.

The activity of GR was determined according to Carlberg and Mannervik [59] with minor modifications. The reaction mixture contained 0.5 mL of 2 mM oxidized glutathione (GSSG), 0.1 mL potassium phosphate buffer (50 mM, pH adjusted at 7.5 with 2 M HCl or NaOH), 0.2 mL of 3 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 0.1 mL of 2 mM NADPH, and 0.1 mL supernatant. The absorbance was measured at 420 nm.

According to Mazhoudi, *et al.* [60] method with slight modifications, the guaiacol peroxidase (GPX) activity was estimated. The reaction mixture containing 0.5 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.2 mL of 1% (m/v) guaiacol, 0.2 mL OF 0.4% (v/v) H_2O_2, and 0.1 mL of the enzyme extract from each sample. Changes in the absorbance were measured at 470 nm over 3 min.

The other sensitive, accurate, low sample concentration and low-cost biochemical analysis, (DPPH), was determined by Blois [61]. The reaction mixtures include 1 mL of 0.5M DPPH to different concentrations of sample and incubated for different times before measuring absorbance at 525 nm. DPPH assay is based on scavenging capability measurement. The nitrogen atom contains an old electron which is reduced by delivering a hydrogen atom from antioxidants to hydrazine.

The total antioxidant capacity was measured according to the procedure of Prieto, *et al.* [62]. The method includes a homogenate sample with the following 0.25 mL 0.6 M sulfuric acid, 0.5 mL 28 mM sodium phosphate, and 0.25 mL 4 mM ammonium molybdate, then incubate at 95˚C for 90 min. The absorbance was measured at 695 nm.

2.4. Detoxification Enzymes Activity

By using the method of Gomori [63], the activity of β-esterase was determined. The reaction mixture contained 18 mg of fast blue B salt dissolved in 10 mM potassium phosphate buffer (pH 7.0), 600 µL of 0.113 M β- naphthyl acetate which was dissolved in 50 % acetone. A volume of 240 µL of this solution was mixed with 10 µL of each sample, and the absorbance was measured at 570 nm over 3 min.

2.5. Biomolecules Concentration

Spectrophotometrically, the total protein concentration of samples was determined by Bradford [64] method. Briefly, 0.9 mL of the dye reagent (10 mg COBB + 5 mL methanol + 10 mL 85% O-phosphoric acid, completed to 100 mL with distilled water) were added to 0.1 mL of each sample in a separate test tube. The contents of the tube were mixed by gentle shaking and left to stand for 2 min. The OD of the protein sample was measured at 595 nm. 2% albumin concentration was used as a standard.

2.6. Statistical Analysis

The effect of both control treatment and contaminated Cu^{2+} treated samples on the oxidative stress parameters levels of organic waste, 5th instars larvae of *H. illucens*, and excreta were assessed by performing *Kruskal-Wallis H (P<0.05)*. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.).

3. Results

3.1. Reactive Oxygen Species and Oxidative Damage

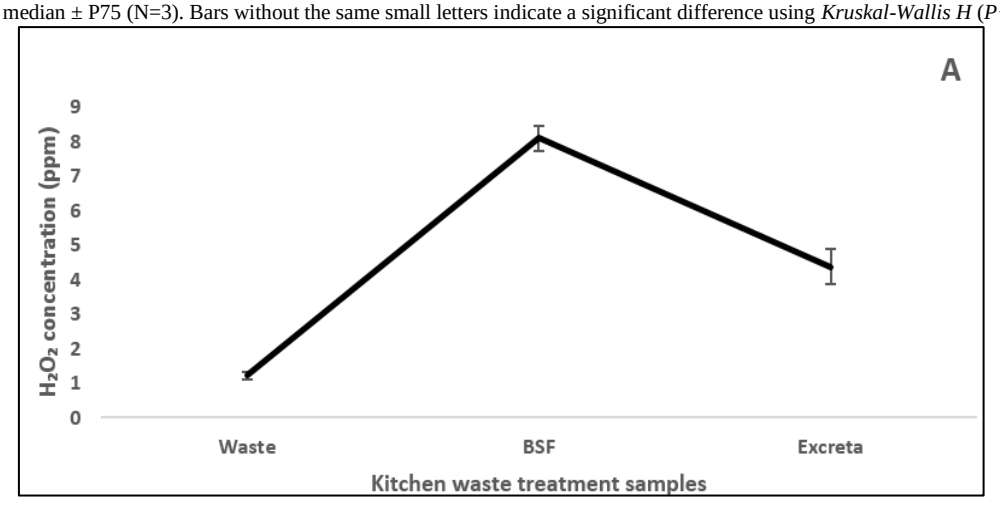
The levels of H_2O_2, PC and LP were generally lower in control than Cu^{2+}-treated KW or VW waste samples (Table 1 and Fig. 1). In control samples, elevated levels of H_2O_2 were observed in the larval excreta than larvae and input organic waste materials. The H_2O_2 increased folds were 10.4 and 17.5-x in the larval excreta; 5.4 and 2.9-x in insect larvae for kitchen and vegetable waste, respectively (*P value < 0.05*) (Table 1). While, the H_2O_2 levels in the Cu^{2+}-treated samples showed an increased level within the larval with 6.5, and 6-x fold than VW and KW, respectively. The concentration of H_2O_2 was increased in the kitchen waste than vegetable input waste. However, the insect and excreta output samples showed an elevation level of ROS in vegetable treated samples, compared with samples collected from the kitchen waste pool. The protein carbonyls (PC) amount in the control samples showed a uniform decrease pattern along with input (KW), process (insect larvae), and output (larval EX), while the VW-treatment showed an accumulation pattern of PC inside insect larvae than VW and EX. In Cu^{2+}-treated samples, there was a significant decrease of PC levels in the EX from the KW treatment samples (Fig. 1C), however, the VW treatment samples showed no significance in the PC amount among VW, BSFL, and EX (*P>0.05*) (Fig. 1D). However, the Cu^{2+}-treated VW samples showed a slightly significant increase inside BSFL than VW and EX (Fig. 1F). While in the KW treatment, the results showed uniform stability between KW, BSFL, and EX (Fig. 1E). The protein carbonyls amount in 5th instar larvae of *H. illucens* feed on kitchen waste were significantly higher in relation to those feed on vegetable waste, however, lipid peroxide BSF feed on vegetable waste showed a higher concentration than those feed on kitchen waste (Fig. 1). In the control treatment, the significant decrease level of lipid peroxides (LP) levels within the insect larvae were shown in both KW&VW than the waste or EX (Table 1).

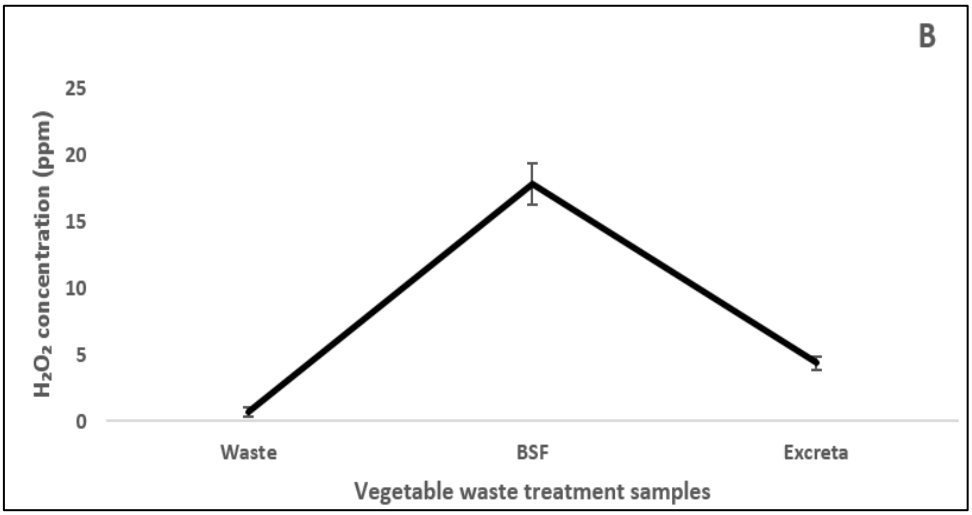
Table-1. oxidative stress parameters (OSP): hydrogen peroxide (H_2O_2), protein carbonyls (PC), lipid peroxides (LP), catalase (CAT), superoxide dismutase (SOD), gluthathione peroxidase (GPX), 2,2 diphenyl-1-picylhydrazyl (DPPH), total antioxidant ability (TAA); β-esterase (β-EST); and total amount of protein (TAP) as median and P75 of the kitchen (KW) or vegetable waste (VW), black soldier fly larvae (BSFL) and BSFL excreta (EX) collected from the media of 7-day organic waste (KW &VW) treated with distilled water (1 gm/10 mL)

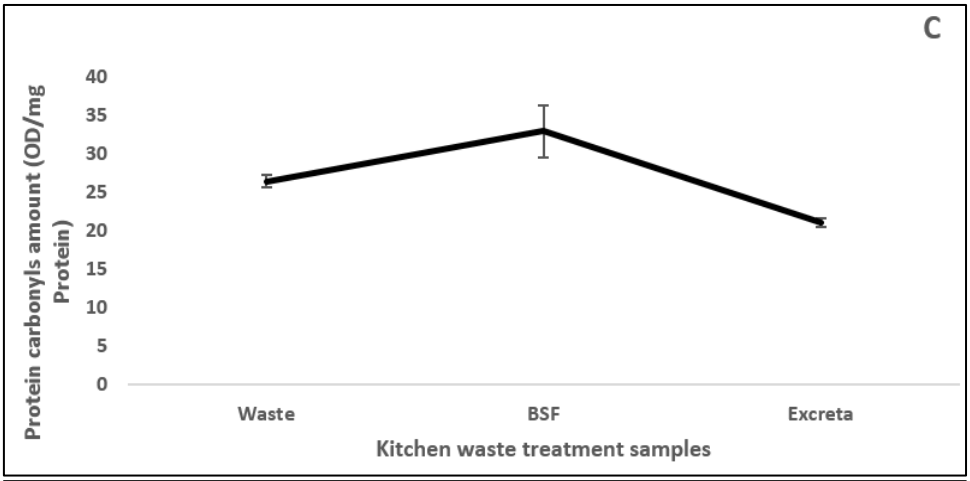
OSC	OSP	Input				Insect (process)				Output			
		KW		VW		KW/BSFL		VW/BSFL		KW/EX		VW/EX	
		Median	P75	Median	P75	Median	P75	Median	P75	Median	P75	Median	P75
ROS	H_2O_2	0.19	0.04	0.13	0.02	3.12	0.91	4	1	2.00	1.20	2.30	0.15
OD	PC	20	2.08	9.51	1.04	6.31	0.41	9.80	0.41	7.21	2.10	9	0.42
	LP	11	1	9.50a	0.25	3.12	0.21	8.43	0.20	8.11	2.61	9.50a	0.30
EA	CAT	0.22	0.02	0.31	0.02	0.94	0.02	1.81	0.15	0.99	0.11	1.40	0.25
	SOD	1.60	0.15	0.23	0.02	0.11	0.002	0.26	0.05	0.12	0.001	0.17	0.03
	GPx	2	0.15	0.35	0.04	0.84	0.21	1.41	0.15	0.11	0.02	0.14	0.02
NEA	DPPH	9	1.50	19	3	10	1.10	32	2.50	1.42	0.21	2	0.60
	TAA	0.12	0.02	0.06	0.01	0.10a	0.001	0.13	0.03	0.13a	0.001	0.15	0.01
DE	β-Ease	0.10a	0.01	0.24b	0.01	0.11a	0.01	0.23b	0.02	0.99	0.002	1.40	0.10
BC	TAP	3.91	0.15	2.50	0.45	39	0.25	45	0.38	8.12	0.51	9.11	0.90

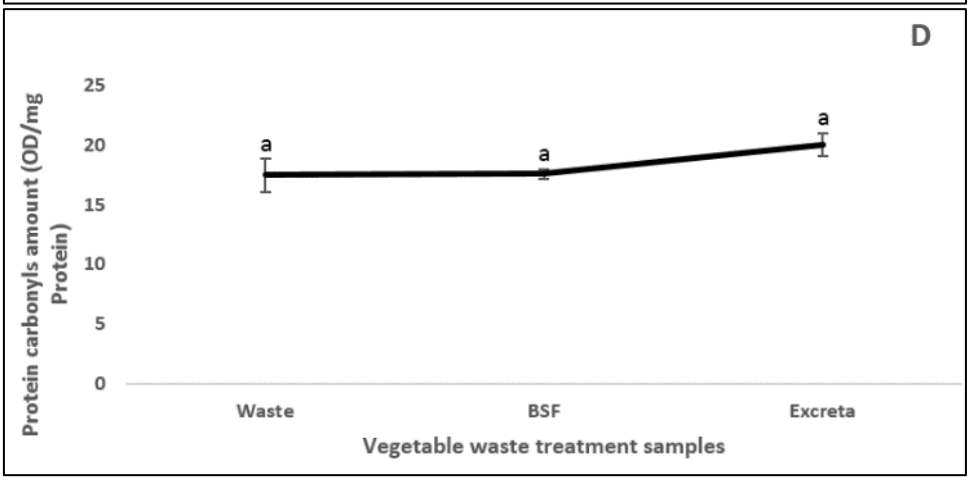
OSC: oxidative stress category; OD: oxidative damage; EA: enzymatic antioxidants; NEA: non-enzymatic antioxidants; DE: detoxification enzymes; BC: biomolecules concentrations.

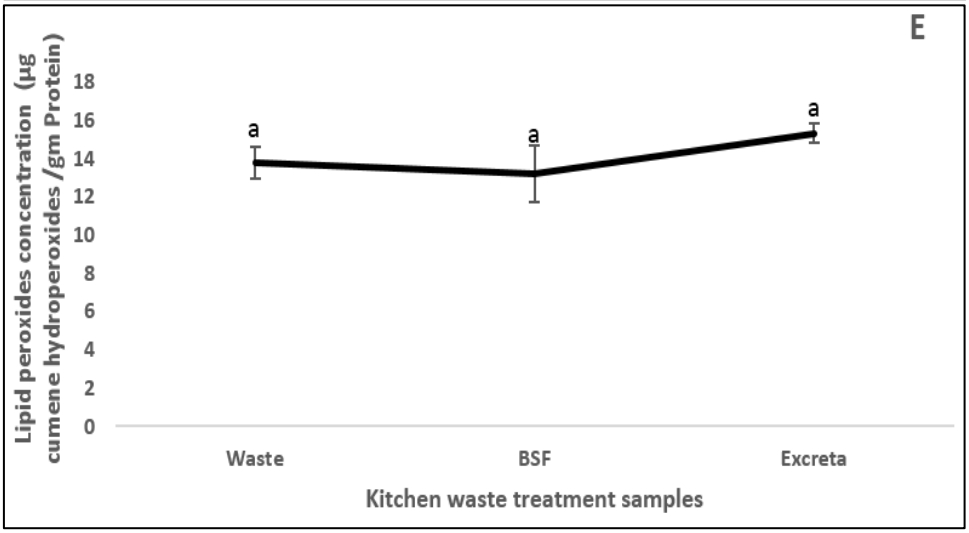
Fig-1. The Reactive oxygen species (ROS) concentration, Hydrogen peroxide (H_2O_2) (A&B) and oxidative stress damage, protein carbonyls amount (C&D), and lipid peroxide concentration (E&F) of kitchen (KW) (A,C&E) or vegetable (VW) (B,D&F) wastes, 5th instar larvae *H. illucens*, and larvae excreta collected from the media of 7-day organic waste (KW&VW) treated with (1 gm:10 mL) 100 mg/mL Cu^{2+}. Values represented as median ± P75 (N=3). Bars without the same small letters indicate a significant difference using *Kruskal-Wallis H* (*P<0.05*)

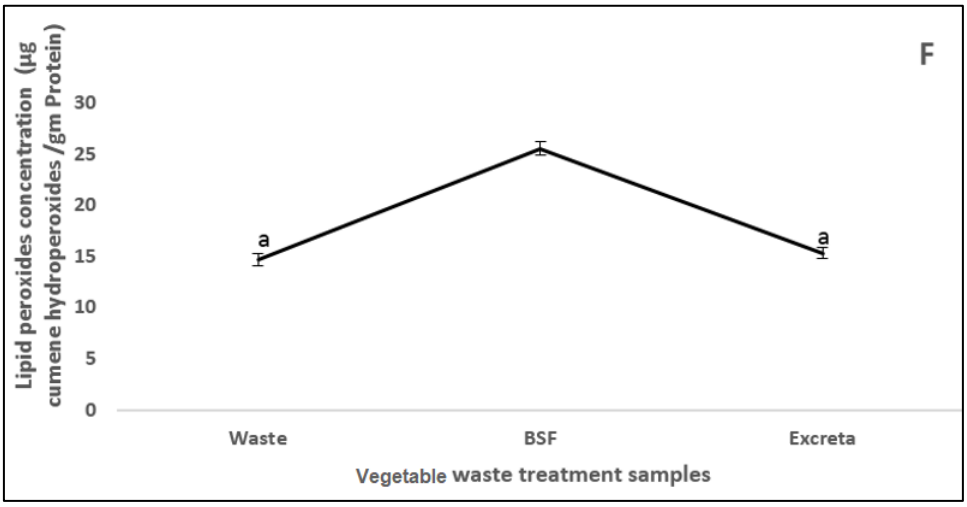



B
H_2O_2 concentration (ppm)
25
20
15
10
5
0
Waste
BSF
Excreta
Vegetable waste treatment samples


C
Protein carbonyls amount (OD/mg Protein)
40
35
30
25
20
15
10
5
0
Waste
BSF
Excreta
Kitchen waste treatment samples


D
Protein carbonyls amount (OD/mg Protein)
25
20
15
10
5
0
a
a
a
Waste
BSF
Excreta
Vegetable waste treatment samples

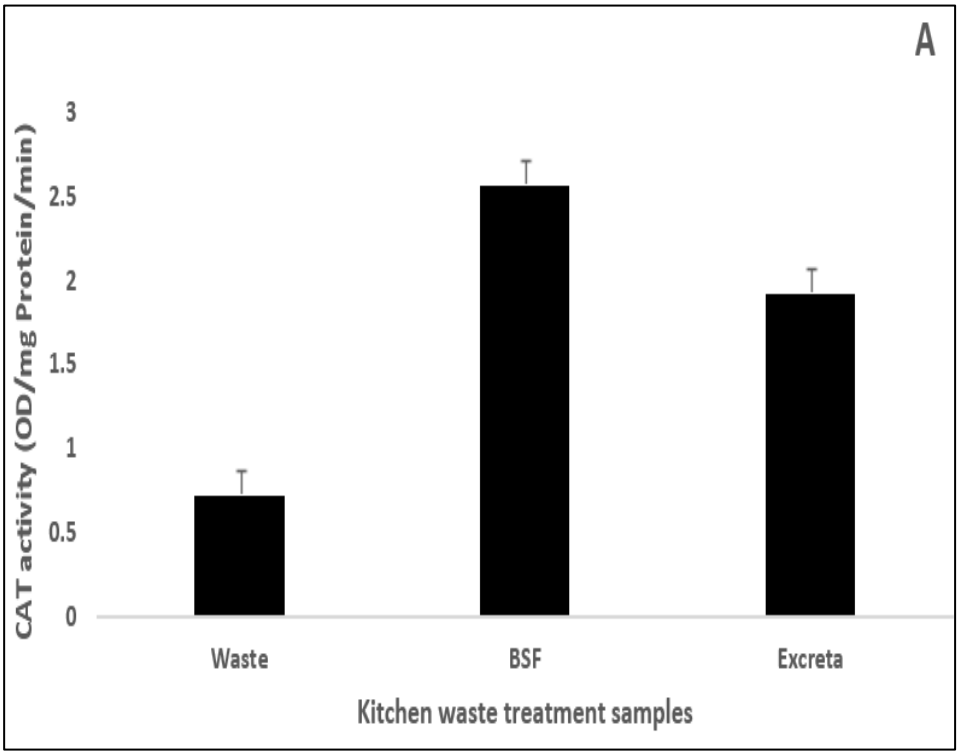

E
Lipid peroxides concentration (µg cumene hydroperoxides /gm Protein)
18
16
14
12
10
8
6
4
2
0
a
a
a
Waste
BSF
Excreta
Kitchen waste treatment samples

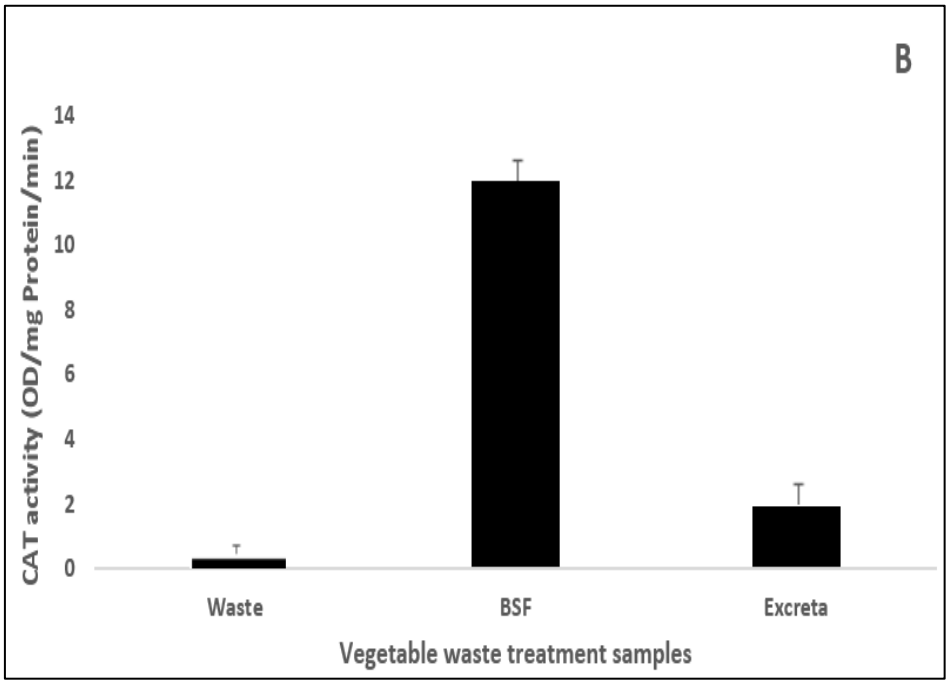


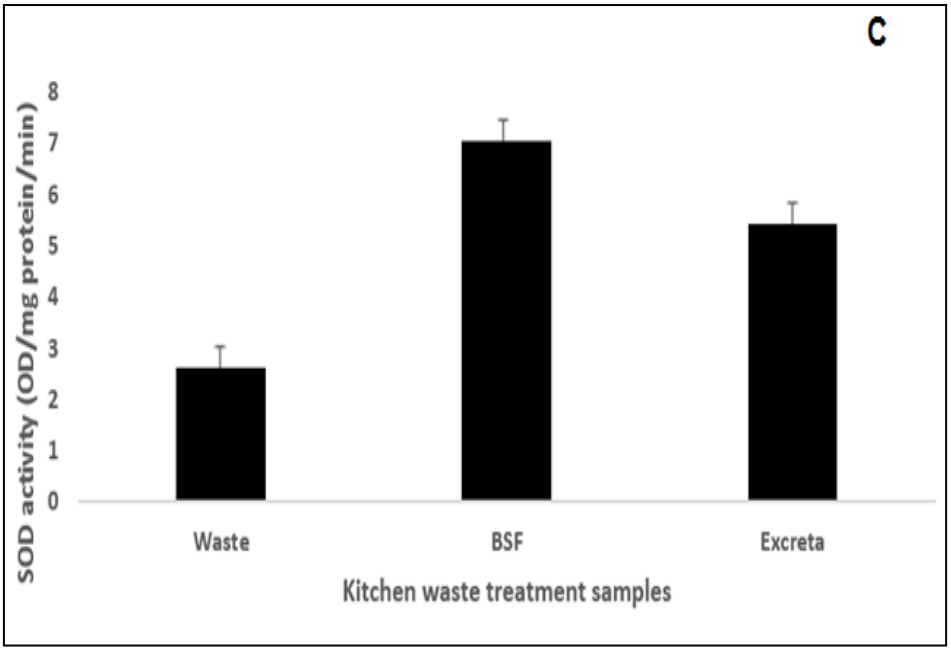
3.2. Enzymatic and Non-Enzymatic Antioxidants Response

The enzymatic and non-enzymatic antioxidants response showed a significant decrease inside the control samples than Cu^{2+}-treated samples in both KW and VW (Table 1, Figs. 2, 3). In control samples, unlike Cu^{2+}-treated samples, each antioxidant showed a unique pattern among wastes, insects, and excreta. For instance, the constitutive CAT activity of KW and VW were significantly decreased than BSFL and EX, while the constitutive activity of SOD and GPx of KW were significantly increased than insect larvae and excreta. However, the non-enzymatic DPPH levels of BSFL showed significantly increased levels than waste and excreta in both KW&VW. In kitchen waste treatments, the non-enzymatic TAA showed a significant decrease inside BSFL than waste and excreta, while the excreta from VW treatments showed a significant increase than other samples ($P<0.05$) (Table 1). The enzymatic activity and non-enzymatic levels of Cu^{2+}-treated samples showed a uniform trend pattern in both KW and VW samples (Figs. 2, 3). As the antioxidant's levels inside BSF were significant increase than waste and excreta. This trend occurred in CAT, SOD, GPx, DPPH, and TAA in both KW and VW (Fig. 1A- D, F and Fig. 2A-D), except the case of GPx activity in the KW, the GPx activity in the KW was significantly increased than the other samples ($P<0.05$) (Fig. 2E). The activity of the three principal antioxidant enzymes (CAT, GPx, and SOD) and non-enzymatic levels showed that 5th instar *H. illucens* feed on vegetable waste higher than those feed on kitchen waste (Figs. 2, 3).

Fig-2. The enzymatic antioxidants response catalase (CAT) (A&B), superoxide dismutase (SOD) (C&D), and glutathione peroxidase (GPx) (E&F) of kitchen (KW) (A,C&E) or vegetable (VW) (B,D&F) wastes, 5th instar larvae *H. illucens*, and larvae excreta collected from the media of 7-day organic waste (KW&VW) treated with (1 gm:10 mL) 100 mg/mL Cu^{2+}. Values represented as median ± P75 (N=3). Bars without the same small letters indicate a significant difference using *Kruskal-Wallis H* ($P<0.05$)




B
CAT activity (OD/mg Protein/min)
14
12
10
8
6
4
2
0
Waste
BSF
Excreta
Vegetable waste treatment samples


C
SOD activity (OD/mg protein/min)
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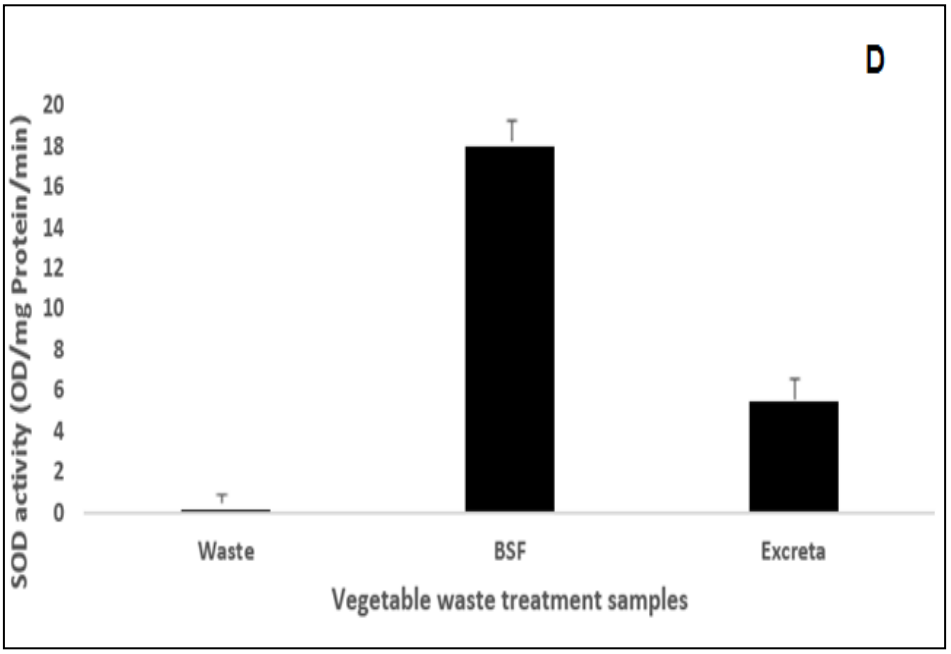
7
6
5
4
3
2
1
0
Waste
BSF
Excreta
Kitchen waste treatment samples


D
SOD activity (OD/mg Protein/min)
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18
16
14
12
10
8
6
4
2
0
Waste
BSF
Excreta
Vegetable waste treatment samples

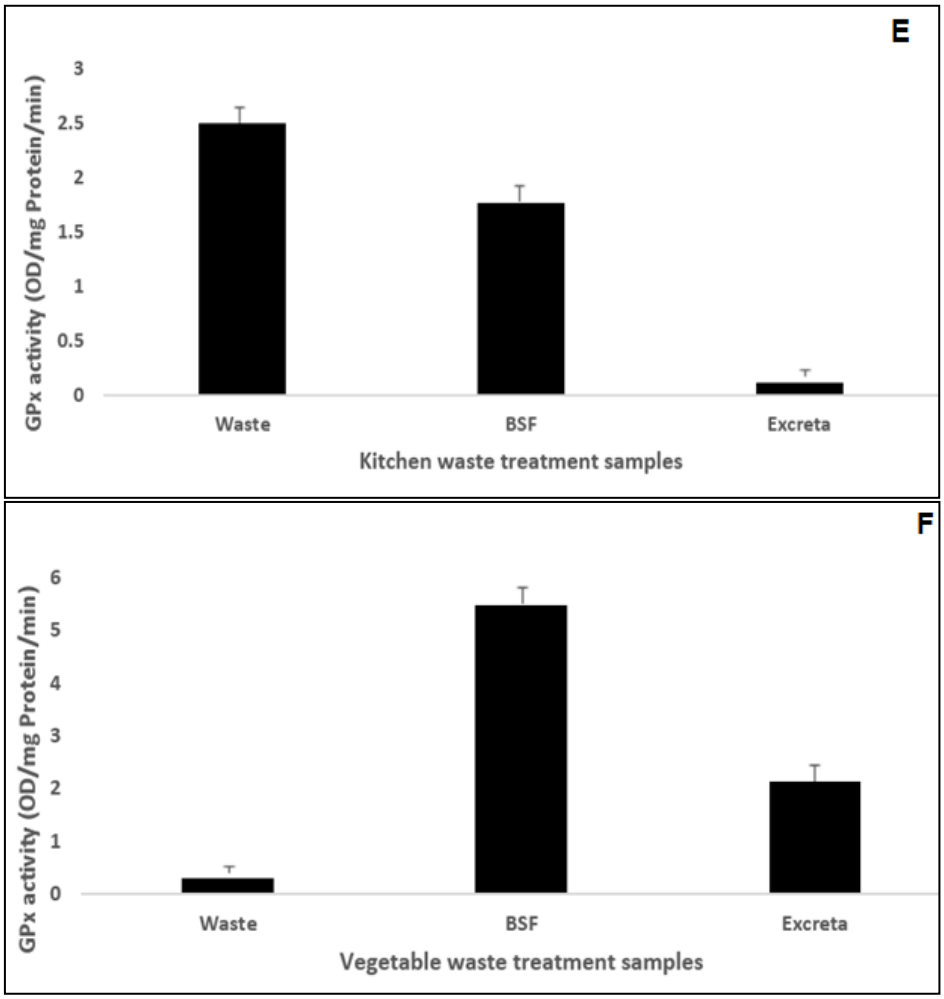
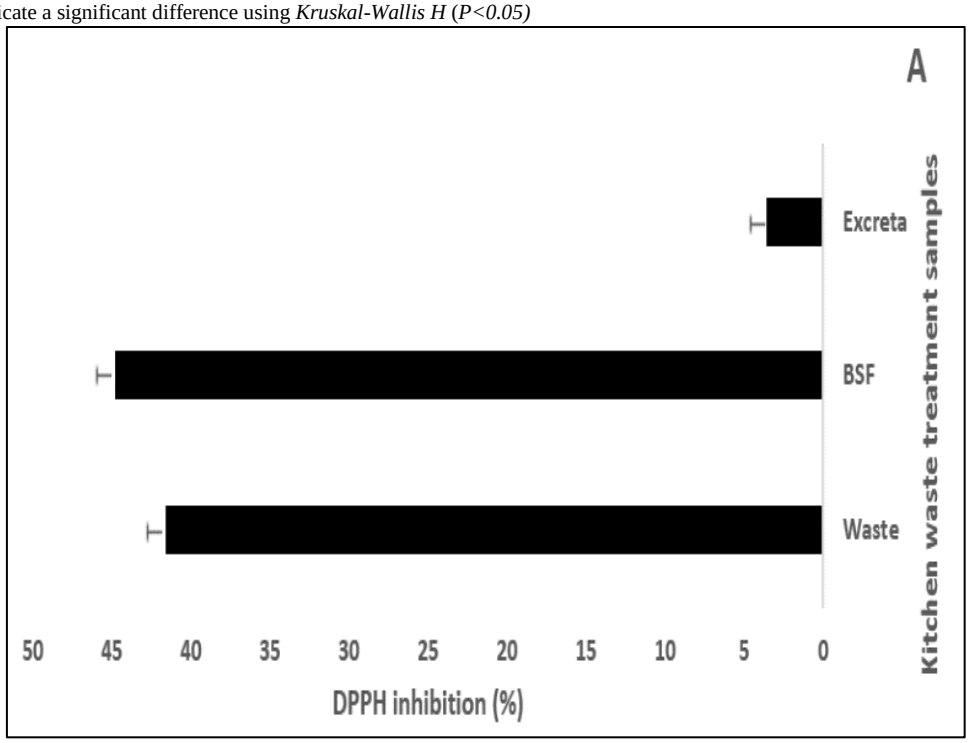
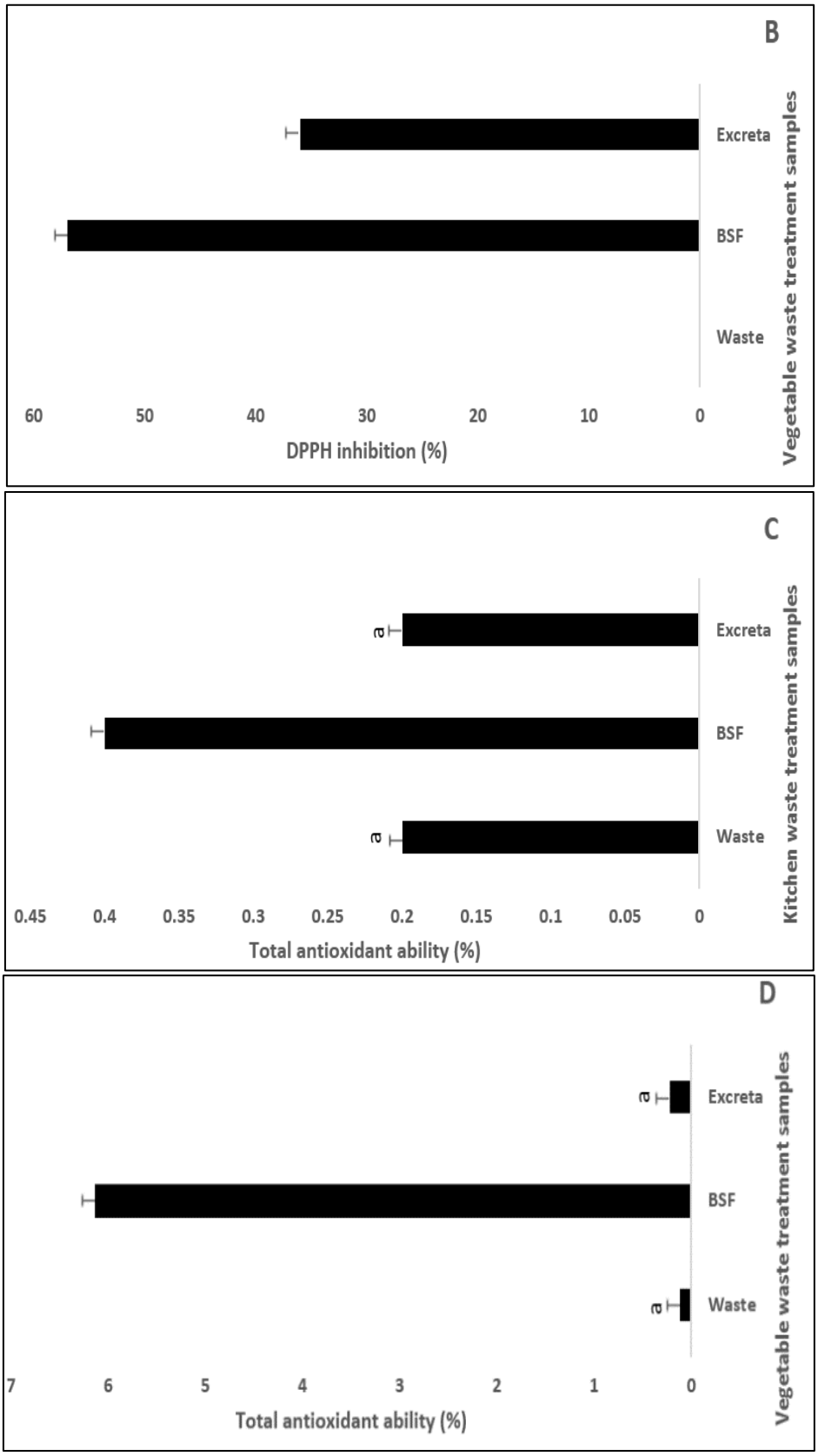


Fig-3. Inhibition percentage of non-enzymatic antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH)) (A&B) and total antioxidant ability (TAA) (C&D) of kitchen (KW) (A&C) or vegetable (VW) (B&D) wastes, 5th instar larvae *H. illucens*, and larvae excreta collected from the media of 7-day organic waste (KW&VW) treated with (1 gm:10 mL) 100 mg/mL Cu^{2+}. Values represented as median ± P75 (N=3). Bars without the same small letters indicate a significant difference using *Kruskal-Wallis H* (*P<0.05)*





3.3. Detoxification Enzyme Activity and Biomolecules Concentration

The constitutive detoxification enzyme activity (β-esterase) showed a different pattern in VW compared to KW treated samples. The β-esterase activity of BSFL was significantly increased than KW and EX; however, the depletion levels of the detoxification enzymes inside the insect larvae occurred compared to VW and excreta (Table 1). Yet, the concentration of biomolecules, protein concentration, has a uniform pattern in the KW and VW treatment samples. The results showed a significant increase in the excreta than BSFL and waste (Table 1). In Cu^{2+}-treated samples, both activities of detoxification enzymes and biomolecules concentration showed a significant increase in the excreta than input waste and insect for VW and KW (Figs. 4, 5). Yet there was no significant difference among VW and BSF in the activities of β-esterase (Fig. 2B). Also, the non-significance difference among BSF and KW or VW has occurred in the concentration of total protein (Fig. 5A, B). The level of β-esterase activity

in BSF feed on kitchen waste had higher activity than those feed on vegetable waste (Fig. 4). Yet, the total protein amount didn`t reveal significant differences in BSF feed on kitchen waste or vegetable waste (Fig. 5).

Fig-4. Detoxification enzyme activity , β-esterase (A&B) of kitchen (KW) (A) or vegetable (VW) (B) wastes, 5th instar larvae *H. illucens*, and larvae excreta collected from the media of 7-day organic waste (KW&VW) treated with (1 gm:10 mL) 100 mg/mL Cu^{2+}. Values represented as median ± P75 (N=3). Bars without the same small letters indicate a significant difference using *Kruskal-Wallis H* (*P<0.05)*

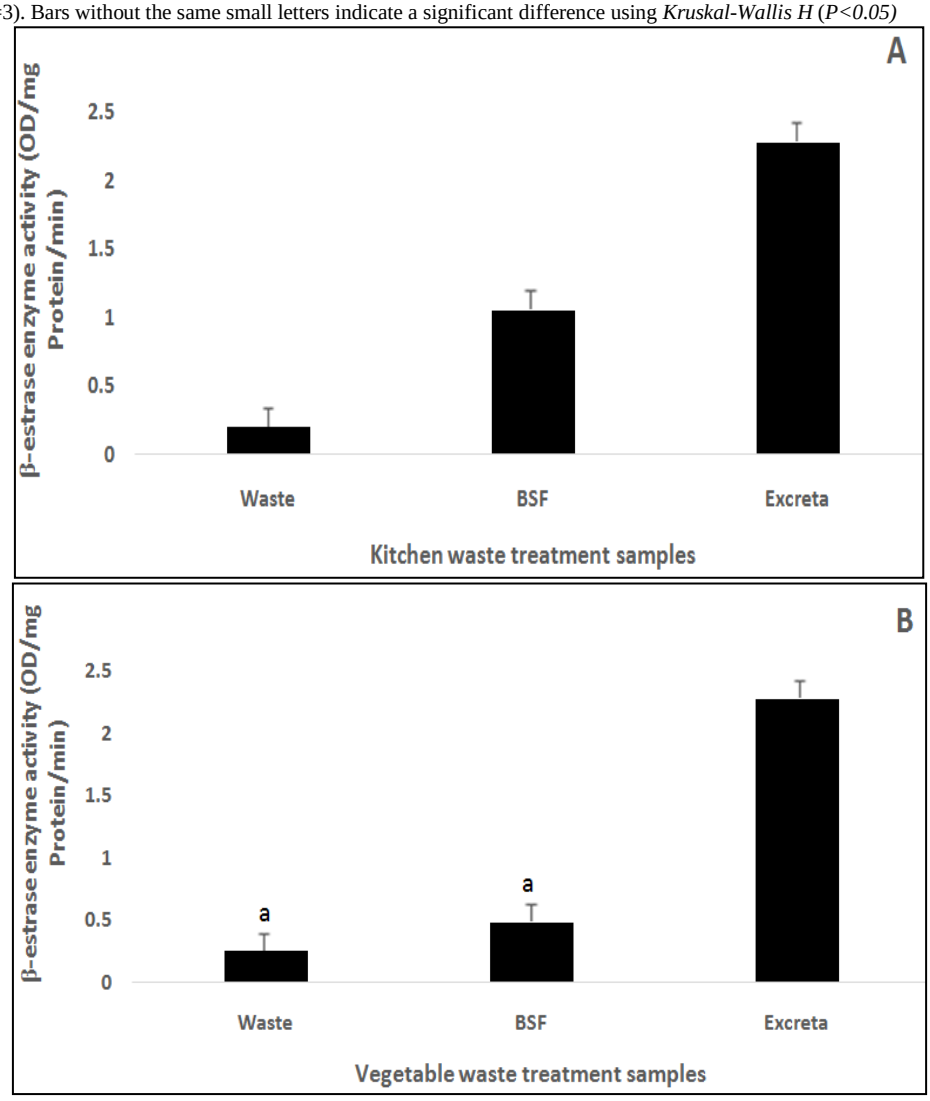
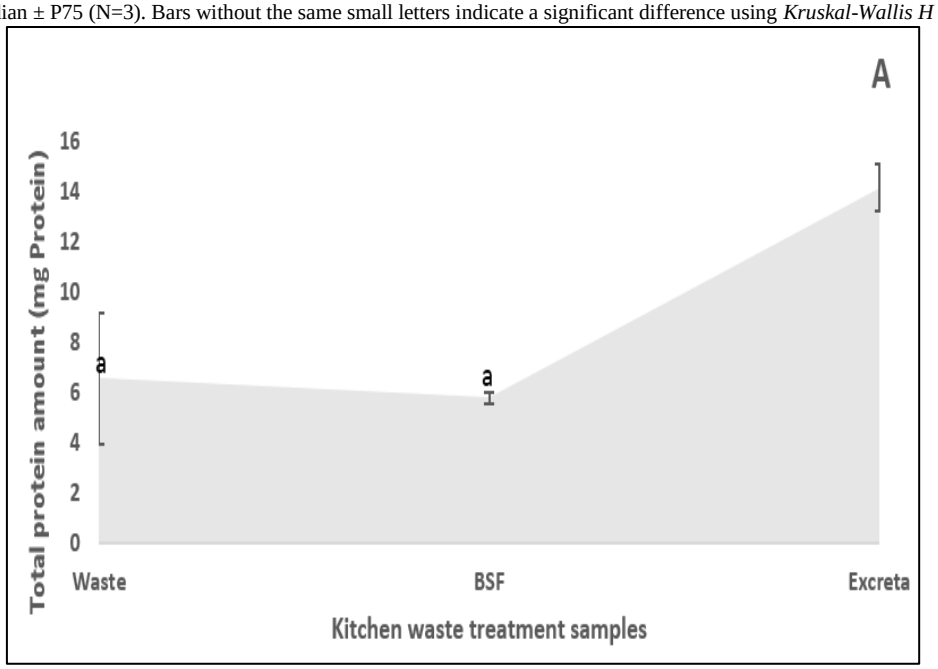
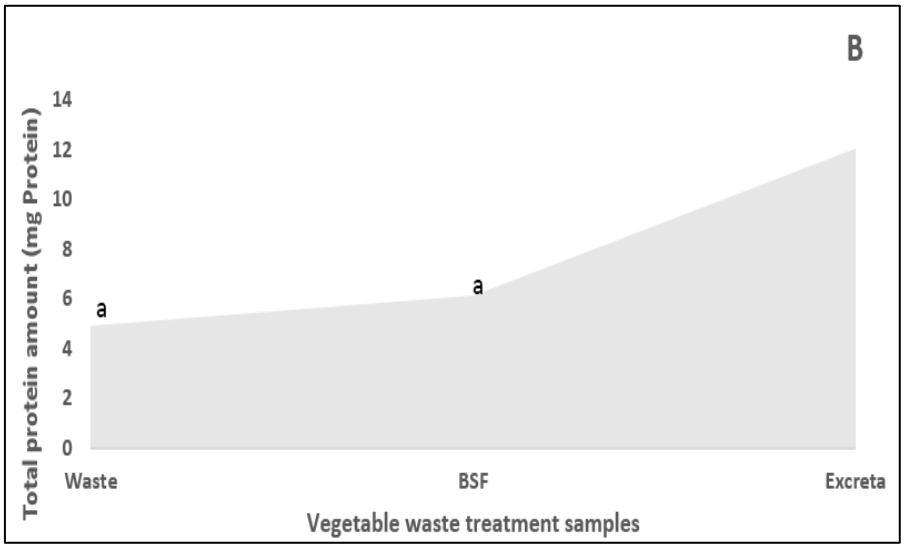


Fig-5. Biomolecules concentration , total amount of proteins (TAP) (A&B) of kitchen (KW) (A) or vegetable (VW) (B) wastes, 5th instar larvae *H. illucens*, and larvae excreta collected from the media of 7-day organic waste (KW&VW) treated with (1 gm:10 mL) 100 mg/mL Cu^{2+}. Values represented as median ± P75 (N=3). Bars without the same small letters indicate a significant difference using *Kruskal-Wallis H* (*P<0.05)*





4. Discussion

The problem of dealing with estimation the oxidative stress factors, or more specifically, the mechanisms of using oxidative stress, are considered as the main point of interest to scientists dealing with phenomena of bioremediation, toxicology, and adaptation. In recent studies, these researches topics have gained significant value, mainly due to the increased stress levels as a result of the human and environments-related activities pressure [21, 65]. These abnormal changes require organisms' adaptation and mitigation. These responses can be vital as they can allow the studying of specific defense mechanisms against various and different stress factors [2, 5, 7, 8, 14].

Organic waste accumulation and contamination have a serious problem with ecosystem function and structure [65-68]. Accumulation of organic waste is dealing with the quantity of organic waste production. As, within the early decades of this century, annual organic waste production was over 600 million tones, and by 2025, it is expected to reach around one billion tones [69]. The Landfill is currently the most common method for disposing of organic waste, which has the potential to contribute to global warming as well as soil and water pollution, making it an increasing problem for society Christensen, *et al.* [70]. Otherwise, organic waste contamination, especially heavy metals, can be a source of environmental pollution as a result of oxidative stress magnification inside the various and surrounding living organisms [71-73].

Exposure to environmental pollutants, including organic waste, chemical pollutants, and heavy metals, is linked with the elevated levels of oxidative stress through increasing the production of ROS; which cause deleterious effects on the macromolecules of the living organisms [2, 3, 5, 7, 8, 14, 65]. Besides that, heavy metals, especially Cu^{2+}, can increase ROS production by disturbing the respiratory metabolism rate [74]. The imbalance between oxidants and antioxidants can lead to oxidative damage products [75-77]. So, the use of insect to convert organic waste into biomass, which can then be used for an innovative purpose, were studied in various studies [65, 78-85], however, the ability to use insect as a bioremediation agent of pollutants contamination is still needed more studies and clarifications mechanisms [86, 87].

In the present study, the oxidative stress parameters, detoxification enzymes activity and biomolecules concentration of the control and Cu^{2+} contaminated organic waste (kitchen waste and vegetable waste) on the input organic waste, insect 5^{th} instar larvae, *Hermetia illucens*, and insect excreta were studied. Although the insect larvae fed on the control organic waste, the levels of H_2O_2 don't tend to zero value (Table 1). This may be due to the necessity of maintaining the optimal concentration of H_2O_2 in cells as it can be used in intracellular signalling and regulation [88]. However, the interactions between the organic waste and heavy metals especially Cu^{2+} was not clear, some differences might come from the different chemical compositions of the organic waste, such as organic matter, which can affect the ability to uptake the other chemical pollutants [89-91].

The organic waste type includes vegetable waste, food waste, plant tissues waste, animal offal, vegetable waste, restaurant waste and animal manure [65, 92, 93] can lead to oxidative stress especially when contained chemical pollutants, as arsenic [94] as a result of ROS production [95, 96]. In the present work, the levels of oxidative stress were evaluated directly and indirectly to evaluate the ability of BSFL to bioremediate the Cu^{2+}-organic waste contamination. These oxidative stress parameters were evaluated, also, for control organic waste through assessment of the H_2O_2 levels, macromolecules damaged products (protein carbonyls and lipid peroxides), as well as measuring the enzymatic and non-enzymatic antioxidants levels (SOD, CAT, GPx, DPPH or TAA), besides that the biomolecules concentration (proteins) and related detoxification enzymes in the KW or VW, 5^{th} instar larvae of BSF and larval excreta. These were done according to the generally accepted previous studies [7, 65, 97, 98].

The results showed a significant decrease in the H_2O_2 levels, as well as macromolecules oxidatively damaged products, protein carbonyls and lipid peroxides levels, in the control samples than Cu^{2+}-treated samples in both kitchens and vegetables waste treatment samples (Table 1 & Fig. 1). Our results are similar to other studies which showed that copper exposure leads to a stress-dependent production of ROS which leads to protein carbonylation and lipid peroxidation elevation [99, 100]. The studies of Baryla, *et al.* [101] and Lushchak [102] emphasized the use of lipid peroxides levels as an indicator of the oxidative stress parameters, moreover, the study of Ahmed [32] &

Yousef, *et al.* [96] revealed that protein carbonyls amount, as well as lipid peroxides levels, can be used as an indicator of oxidative stress in *Aedes caspius* and *Aiolopus thalasinus*, respectively. Also, Zaman, *et al.* [94] showed a significant increase in the lipid peroxides and protein carbonyls amount as a result of treatment of adult female house flies, *Musca domestica*, and 4th-instar cabbage loopers, *Trichoplusia ni with* As^{3+}, ($NaAsO_2$) and As^{5+}, (Na_2HAsO_4).

Also, Copper can catalyze the production of ROS, such as superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$), through Fenton and Haber-Weiss reactions. ROS can react with lipids, proteins, or DNA molecules and can initiate chain reactions that engage lipid peroxidation, protein oxidation and fragmentation, and DNA strand breaks [103]. The obtained results approved that the proteins are the most important targets of free radical attack in the cells [102]. This phenomenon occurred as ROS can convert amino groups of proteins and cause changes in the protein structure and function. Therefore, oxidative stress correlates with several modified carbonyls groups of protein damage [103]. The proteins damage leads to conformation disruption of protein molecules, including enzymes [104, 105]. Besides that, the significant elevation of lipid peroxides occurred when the antioxidants are depleted by the actions of ROS [5, 7, 8, 96, 106].

The present work showed the elevation production of ROS in the larval excreta than larval tissues (Table 1) which confirm the criteria of the ability of the insect to detoxify toxicants through the action of antioxidants, excretion [9, 107]. However, in Cu^{2+}- treatment samples, the elevated levels of H_2O_2 inside the BSF 5th instar larvae than the other samples in both kitchen and vegetables waste treatment, indicate the ability of the insect to store the toxicants and ROS [47]. Additionally, the present study showed the depletion levels of protein carbonyls amount in the kW treatment samples than VW, however, the elevated levels of both enzymatic and non-enzymatic antioxidants in the VW than KW in both control and Cu^{2+}-treated samples (Table 1; Figs. 1, 2, 3). These results are following previously accepted knowledge that deals with the use of vegetables as input waste to produce BSFL and frass. These BSFL products are comparable to other products and are considered valuable and sustainable resources for the agricultural industry [108].

The action of toxicants and oxidants may lead to an increase in the levels of non-enzymatic antioxidants, such as DPPH or TAA as well as the activity of antioxidants and detoxification enzymes such as SOD, CAT, GPx, and β-esterase, respectively. Moreover, SOD and CAT are considered as key antioxidant enzymes responsible for ROS scavenging [106], while, GPx can overcome the low concentration of ROS as, H_2O_2 [96]. Also, esterases are considered as a vital detoxification enzyme in various insects' species such as *Hermetia illucens* (unpublished data), *Blattella germanica* [109], *Culex quinquefasciatus* [110], and *Trichoplusia ni* [111]. However, DPPH radical scavenging can practice for the antiradical properties assessment of multiple compounds [112]; as well as it can be occurred by the action of antioxidants through donating a hydrogen atom to terminate the free radical chain. Also, Aromatic amino acids like phenylalanine and tyrosine can stabilize DPPH free radicals by donating a proton to electron-deficient free radicals [113]. Additionally, β-esterase is an important component of insect defense against xenobiotic chemicals, such as pesticides [114]. The role of the insect's detoxification system is one of the mechanisms behind the effects of secondary metabolites on pesticide susceptibility [115]. Yet, the total antioxidant capacity can assess the ability of all antioxidants in the living organisms to neutralize the oxidation effects of oxidants [116], so it can be used as a tool for evaluating the redox status [117].

The results of the present study emphasized that the redox-active metals could increase the levels of antioxidants and detoxification enzymes, as SOD, CAT, GPx, β-esterase, DPPH radical scavenging, and TAA compared with control samples value (Table 1; Figs. 2, 3, 4). The same observation was showed in the Zaman, *et al.* [94] study which approved that SOD, CAT, glutathione-s-transferase (GST), the peroxidase activity, glutathione reductase (GR), lipid peroxidation and protein oxidation levels were induced by As^{3+} in *M. domestica*, however, CAT and GSTPX were not affected. Additionally, As^{5+} did not affect *M. domestica*. So, the suggestion of using Cu^{2+} and As^{3+} as a pro-oxidant was proposed especially for *H. illucens* and *M. domestica*, respectively. Additionally, the study of Tuncsoy, *et al.* [100] showed a significant elevation of SOD, CAT, GPX, GST, acetylcholine esterase after treatment with copper oxide nanoparticles with *Galleria mellonella.* Also, the elevation of total antioxidant capacity, protein carbonyl content and activities of SOD, CAT, POX or GST of the *Helicoverpa armigera* adults after UV light exposure for 30 min were tested and approved the increased oxidative stress as a result of exogenous factors. Meng, *et al.* [118], suggested that the elevation activity of SOD and CAT in the cells after detoxification may appear due to increased ROS generation. Briefly, SOD can dismutase the superoxide anion radical and CAT [119] or GPx [120] can decompose H2O2 at high or low levels, respectively [5, 96]. All these findings can answer the 3rd question of our hypothesis which is related to the ability of BSFL to produce more antioxidants, especially within contaminated or polluted zones.

Yet, the SOD antioxidant enzyme activities were suppressed in *Trichoplusi ni*, by 29.4% as a result of treatment with As^{5+} [94], as well as SOD depletion occurred in *Galleria mellonella* after treatment with 10 µg Cu/L of CuO [100]. Also, the reduction of glutathione reductase activity and total thiol (SH) content of *Scenedesmus* sp after Cu^{2+} and Zn^{2+} treatment [121]. These findings may link with the present results related to depletion of total protein content after treatment of Cu^{2+} (Fig. 5) as the enzymes are protein in nature.

Additionally, free radical scavenging is generally done by the action of redox status through Fenton reaction [5]. As well, the study of Kaur, *et al.* [122] showed that plant gall extract has free radical scavenging to inhibit oxidative damage to biomolecules, briefly, Fe–ascorbate can inhibit lipids peroxidation through termination of the lipid peroxidation chain reaction. Moreover, the study of Oghenesuvwe and Paul [123] mentioned the DPPH antioxidant properties as an insect's isolated compounds product as, *Reticulitermes speratus*, *Tenodera aridifolia, Camponotus obscuripes* [124], *Allomyrina dichotoma* [125], and *Protaetia brevitarsis* [126]. Besides that, the elevated

levels of total protein amount in the insect larvae than input waste emphasized the ability of BSFL to convert the organic waste into bio-based valued products (Table 1). Besides that, the availability of bioactive compounds inside BSF biomass to enhance their nutritive value which can use as animal feed [65, 127, 128].

Many types of organic waste, such as food waste, plant tissues waste, animal offal, and animal manure, have been recommended as effective organisms for turning them into insect biomass [129]. The resultant larval (*H. illucens*) biomass has proved to be a beneficial feed component for pigs, chickens, and fish farmed animals [130]. In addition, BSFs are considered a great source of energy and easily digested amino acids [131]. As the BSFs' primary source of amino acids, the protein composition of the substrate has an impact on their amino acid availability [132]. Overall, BSFs have a higher concentration of essential amino acids [133-135] and a better amino acid profile than soya bean meal and most traditional protein sources [136]. The amount of total protein found in the 5^{th} instar *H. illucens* feed on Cu^{2+} contaminated kitchen waste or vegetable waste revealed no significant differences (Fig. 5).

The previous study investigated the ability of BSF to be used in entomoremediation of heavy metal pollution as it has a strong tolerance to cadmium stress, this was done through identifying and characterizing three *MTs* genes in BSF [137]. This finding supports our results with measuring oxidative stress parameters (Table 1; Figs. 1, 2, 3, 4, 5). These investigations have the answer to one of our questions related to the ability to use the oxidative stress parameters as an indicator of bioremediation ability. Besides that, the present results can emphasize the 1^{st} question in our hypothesis which is related to the capability of management of Cu^{2+}-contaminated organic waste using BSFL, as well as, the study of Diener, *et al.* [138] confirmed the great potential of BSFL as a waste manager in low and middle-income countries, yet, the high percentage of larval mortality due to elevated zinc concentrations in the waste material and anaerobic conditions in the experimental trays may occur.

The research conducted in this study suggests that significant differences in the oxidative stress parameters, macromolecules concentration, and detoxification enzymes of *H. illucens* treated with Cu^{2+}-contaminated organic waste (KW &VW) were evaluated compared to control value. The elevation levels of antioxidants inside the insect than waste and excreta suggest its role as a source of essential products characterized by economic, environmental and social aspects of sustainability. Besides that, the potential use of oxidative stress parameters as a bioremediation ability of BSFL to various environmental toxins. All these findings are matched by previously accepted knowledge [137, 139-141].

Declarations

Ethical Approval and Consent to Participate

This article does not contain any studies with human participants or animals that require ethical approval.

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Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Author Contribution

Bassiony D, Nagah A., Fawzy M. A., Hussein M. Y. Ibrahim H. M., Ibrahim N. Y., Ashry H., Aboelhassan A. M., Mahmoud A. H., Abd El-Monem D. H. performed all the experimental measurements and drafting the first edition of this paper; Abdelfattah, E. were involved in idea clarification, planning of this work, aided in interpreting the results with cited references, and developed the final version of the manuscript and supervised the work. All authors discussed the results and commented on the manuscript.

References

[1] Abdelfattah, E. A., 2016. "Biomolecules oxidation and antioxidant enzymes response as a result of injection of oxidative stressor into 5th instar of Schistocerca gregaria (Orthoptera, Acrididae)." *Entomol Ornithol Herpetol*, vol. 5, pp. 2161-0983.

[2] Abdelfattah, Augustyniak, M., and Yousef, H. A., 2017. "Biomonitoring of genotoxicity of industrial fertilizer pollutants in Aiolopus thalassinus (Orthoptera: Acrididae) using alkaline comet assay." *Chemosphere*, vol. 182, pp. 762-770.

[3] Abdelfattah and Dorrah, M. A., 2015. "L-dopa and ferrous iron increase DNA strand-breaks in the desert locust schistocerca Gregaria (Orthoptera: Acridade)." *EFFLATOUNIA*, vol. 15, pp. 1-7.

[4] Colinet, H., Sinclair, B. J., Vernon, P., and Renault, D., 2015. "Insects in fluctuating thermal environments." *Annual Review of Entomology*, vol. 60, pp. 123-140.

[5] Renault, D., Dorrah, M. A., Mohamed, A. A., Abdelfattah, E. A., and Bassal, T. T., 2016. "Assessment of oxidative stress and activities of antioxidant enzymes depicts the negative systemic effect of iron-containing fertilizers and plant phenolic compounds in the desert locust." *Environmental Science and Pollution Research*, vol. 23, pp. 21989-22000.

[6] Abdelfattah, 2020. "Integration between monitoring and bio-monitoring systems to assessment the impacts of normal levels of environmental pollutants." *Entomol Ornithol Herpetol*, vol. 9, pp. 221-227.

[7] Abdelfattah and Renault, D., 2021. "Effect of different doses of the catecholamine epinephrine on antioxidant responses of larvae of the flesh fly." *Sarcophaga dux. Environ Sci. Pollut. Res.*, Available: https://doi.org/10.1007/s11356-021-16325-9

[8] Abdelfattah, Augustyniak, M., and Yousef, H. A., 2021. "Stage-, sex-and tissue-related changes in H_2O_2, glutathione concentration, and glutathione-dependent enzymes activity in Aiolopus thalassinus (Orthoptera: Acrididae) from heavy metal polluted areas." *Ecotoxicology*, vol. 30, pp. 478-491.

[9] Nassar, M. I., Abd El-Monem, D. H., Youssef, M., Ibrahim, S. M., Mohamed, S. M., Abd-Aldayem, M. S., and Abdelfattah, E. A., 2020. "Bee venom drug potentiality on the macromolecules damage of the larval gut of hermetia illucens (l.),(diptera: stratiomyidae)." *Journal of the Egyptian Society of Parasitology*, vol. 50, pp. 488-493.

[10] Halliwell, B., 1999. "Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and the effects of nutrition." *Mut. Res.*, vol. 443, pp. 37-52.

[11] Georgieva, N. V., 2005. "Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems—a review." *Bulgarian Journal of Veterinary Medicine*, vol. 8, pp. 1-11.

[12] Guo, J., Wang, X., Wang, W., Jia, L., Guo, W., and Wu, G., 2021. "Protective effects of pretreatment with Fe2+, Cu2+, and Rb+ on phoxim poisoning in Silkworm, Bombyx mori." *Journal of Trace Elements in Medicine and Biology*, vol. 68, p. 126844.

[13] Samet, J. M. and Wages, P. A., 2018. "Oxidative stress from environmental exposures." *Current Opinion in Toxicology*, vol. 7, pp. 60-66.

[14] Yousef, H. A., Abdelfattah, E. A., and Augustyniak, M., 2019. "Antioxidant enzyme activity in responses to environmentally induced oxidative stress in the 5th instar nymphs of Aiolopus thalassinus (Orthoptera: Acrididae)." *Environmental Science and Pollution Research*, vol. 26, pp. 3823-3833.

[15] Storey, J. M. and Storey, K. B., 2000. *Environmental stressors and gene responses*. Elsevier.

[16] Linder, M. C., 2013. *Biochemistry of copper*. Springer Science and Business Media.

[17] Danks, D. M., 1989. "Disorders of copper transport." *The Metabolic Basis of Inherited Disease*, pp. 1411-1431.

[18] Feig, A. L. and Lippard, S. J., 1994. "Reactions of non-heme iron (II) centers with dioxygen in biology and chemistry." *Chemical Reviews*, vol. 94, pp. 759-805.

[19] Lopes, C., Herva, M., Franco-Uría, A., and Roca, E., 2011. "Inventory of heavy metal content in organic waste applied as fertilizer in agriculture: evaluating the risk of transfer into the food chain." *Environmental Science and Pollution Research*, vol. 18, pp. 918-939.

[20] Amritha, P. K. and Kumar, P. A., 2019. "Productive landscapes as a sustainable organic waste management option in urban areas." *Environment, Development and Sustainability*, vol. 21, pp. 709-726.

[21] Liu, Awasthi, M. K., Awasthi, S. K., Duan, Y., and Zhang, Z., 2020. "Effects of black soldier fly larvae (Diptera: Stratiomyidae) on food waste and sewage sludge composting." *Journal of Environmental Management*, vol. 256, p. 109967.

[22] Mor, S., Ravindra, K., Dahiya, R. P., and Chandra, A., 2006. "Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site." *Environ Monit Assess*, vol. 118, pp. 435-456.

[23] Sharma, B., Vaish, B., Singh, U. K., Singh, P., and Singh, R. P., 2019. "Recycling of organic wastes in agriculture: an environmental perspective." *International Journal of Environmental Research*, vol. 13, pp. 409-429.

[24] Kadir, A. A., Azhari, N. W., and Jamaludin, S. N., 2016. "An overview of organic waste in composting." In *MATEC Web of Conferences. EDP Sciences.* p. 05025.

[25] Vaish, B., Sharma, B., Srivastava, V., Singh, P., Ibrahim, M. H., and Singh, R. P., 2019. "Energy recovery potential and environmental impact of gasification for municipal solid waste." *Biofuels*, vol. 10, pp. 87–100.

[26] Mata-Alvarez, J., Mace, S., and Llabres, P., 2000. "Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives." *Bioresour Technol.*, vol. 74, pp. 3-16.

[27] Gao, Q., Wang, X., Wang, W., Lei, C., and Zhu, F., 2017. "Influences of chromium and cadmium on the development of black soldier fly larvae." *Environmental Science and Pollution Research*, vol. 24, pp. 8637-8644.

[28] Wang, H., Dong, Y., and Wang, H., 2014. "Hazardous metals in animal manure and their changes from 1990 to 2010 in China." *Toxicological and Environmental Chemistry*, vol. 96, pp. 1346-1355.

[29] Krishnan, N. and Kodrík, D., 2012. *Endocrine control of oxidative stress in insects*. New Jersey, NJ, USA: Wiley-Blackwell. pp. 261-270.

[30] Chaitanya, R. K., Shashank, K., and Sridevi, P., 2016. "Oxidative stress in invertebrate systems." *Free Radicals and Diseases*, pp. 51-68.

[31] Sirivibulkovit, K., Nouanthavong, S., and Sameenoi, Y., 2018. "based DPPH assay for antioxidant activity analysis." *Analytical sciences*, vol. 34, pp. 795-800.

[32] Ahmed, A. M., 2012. "Lipid peroxidation and oxidative protein products as biomarkers of oxidative stress in the autogenous mosquito, Aedes caspius, upon infection with the mosquitocidal bacterium, Bacillus thuringiensis kurstaki." *Pakistan Journal of Zoology*, vol. 44, pp. 525-536.

[33] Bakr, R. F., Abd Elaziz, M. F., El-barky, N. M., Awad, M. H., El-Halim, A., and Hisham, M. E., 2013. "The activity of some detoxification enzymes in Spodoptera Littoralis (Boisd.) larvae (Lepidoptera–Noctuidae) treated with two different insect growth regulators." *Egyptian Academic Journal of Biological Sciences. C Physiology and Molecular Biology*, vol. 5, pp. 19-27.

[34] Das, M. and Dutta, P., 2014. "Status of insecticide resistance and detoxifying enzyme activity of Aedes albopictus population in Sonitpur district of Assam, India." *International Journal of Mosquito Research*, vol. 1, pp. 35-41.

[35] Buico, A., Cassino, C., Ravera, M., Betta, P. G., and Osella, D., 2009. "Oxidative stress and total antioxidant capacity in human plasma." *Redox Report*, vol. 4, pp. 125-131.

[36] EWUIM, S. C., 2013. "Entomoremediation - a novel in-situ bioremediation approach." *Animal Research International*, vol. 10, pp. 1681-1684.

[37] Gerhardt, K. E., Huang, X. D., Glick, B. R., and Greenberg, B., 2009. "Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges." *Plant Science*, vol. 176, pp. 20–30.

[38] Justin, V., Majid, N. M., Islam, M. M., and Abdu, A., 2011. "Assessment of heavy metal uptake and translocation in Acacia mangium for phytoremediation of cadmium contaminated soil." *Journal of Food, Agriculture and Environment*, vol. 9, pp. 588-592.

[39] Badejo, M. A., 2012. "Ecosystem Engineering as a concept: The significance of functional classification of the true engineers." *Environtropica*, vol. 8, pp. 194 – 199.

[40] Badejo, M. A., Tian, G., and Okoh, I. O., 2004. *Litter transformers an ecosystem engineers in sustainable agro ecosystems. In. BADEJO, M. A. and TOGUN, A. O. (Eds.) strategies tactics of sustainable agriculture in the tropics*. Lagos, Nigeria: College Press Ibadan and Enproct Consultants. pp. 86 – 106.

[41] Boze, B. G. V., Hernandez, A. D., Huffman, M. A., and Moore, J., 2011. "Parasites and dung beetles as ecosystem engineers in a forest ecosystem." *Journal of Insect Behaviour*, vol. 25, pp. 352 – 361.

[42] Clark, M., Tepper, K., Petroll, K., Kumar, S., Sunna, A., and Maselko, M., 2021. "Bioremediation of industrial pollutants by insects expressing a fungal laccase." *BioRxiv*, vol. 4, p. 441736.

[43] Ewuim, S. C., Akunne, C. E., and Faniran, O. J., 2012. "The role of soil insects as allogenic ecosystem engineers in Nigeria." *Environtropica*, vol. 8, pp. 187-193.

[44] Halffter, G. and Mathews, E. G., 1966. "The natural history of dung beetles of the subfamily Scarabaeinae (Coleopera: Scarabaeidae)." *Folia Entomologia*, vol. 12, p. 312.

[45] Jones, C. G., Lawton, J. H., and Shachak, M., 1994. "Organisms as ecosystem engineers." *Oikos*, vol. 69, pp. 373-386.

[46] van Gestel, C. A. M., 2012. "Soil ecotoxicology: State of the art and future directions." *ZooKeys*, vol. 176, pp. 275–296.

[47] Martí-Quijal, F. J., Khubber, S., Remize, F., Tomasevic, I., Roselló-Soto, E., and Barba, F. J., 2021. "Obtaining antioxidants and natural preservatives from food by-products through fermentation: A review." *Fermentation*, vol. 7, p. 106.

[48] Barragan-Fonseca, K. B., Dicke, M., and van Loon, J. J. A., 2017. "Nutritional value of the black soldier fly (Hermetia illucens L.) and its suitability as animal feed - a review." *Journal of Insects as Food and Feed*, vol. 3, pp. 105–120.

[49] Lalander, C., Diener, S., Zurbrügg, C., and Vinnerås, B., 2019. "Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (Hermetia illucens)." *Journal of Cleaner Production*, vol. 208, pp. 211–219.

[50] Mertenat, A., Diener, S., and Zurbrügg, C., 2019. "Black soldier fly biowaste treatment – assessment of global warming potential." *Waste Management*, vol. 84, pp. 173–181.

[51] Smetana, S., Schmitt, E., and Mathys, A., 2019. "Sustainable use of Hermetia illucens insect biomass for feed and food: Attributional and consequential life cycle assessment." *Resources, Conservation and Recycling*, vol. 144, pp. 285–296.

[52] Bosch, G., Van Der Fels-Klerx, H. J., De Rijk, T. C., and Oonincx, D. G. A. B., 2017. "Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (hermetia illucens) and yellow mealworms (tenebrio molitor)." *Toxins*, vol. 9, pp. 1–10.

[53] Parodi, A., Van Dijk, K., Van Loon, J. J. A., De Boer, I. J. M., Van Schelt, J., and Van Zanten, H. H. E., 2020. "Black soldier fly larvae show a stronger preference for manure than for a mass-rearing diet." *Journal of Applied Entomology*, vol. 144, pp. 560–565.

[54] Junglee, S., Urban, L., Sallanon, H., and Lopez-Lauri, F., 2014. "Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide." *American Journal of Analytical Chemistry*, vol. 5, p. 730.

[55] Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., and Stadtman, E. R., 1990. "Determination of carbonyl content in oxidatively modified proteins." *Methods in enzymology*, vol. 186, pp. 464-478.

[56] Hermes-Lima, M., Willmore, W. G., and Storey, K. B., 1995. "Quantification of lipid peroxidation in tissue extracts based on Fe (III) xylenol orange complex formation." *Free Radical Biology and Medicine*, vol. 19, pp. 271-280.

[57] Misra, H. P. and Fridovich, I., 1972. "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase." *Journal of Biological chemistry*, vol. 247, pp. 3170-3175.

[58] Aebi, H., 1984. "Catalase in vitro." *Methods in Enzymology*, vol. 105, pp. 121-126.

[59] Carlberg, I. and Mannervik, B., 1985. "Glutathione reductase." *Methods in Enzymology*, vol. 113, pp. 484-490.

[60] Mazhoudi, S., Chaoui, A., Ghorbal, M. H., and El Ferjani, E., 1997. "Response of antioxidant enzymes to excess copper in tomato (Lycopersicon esculentum, Mill.)." *Plant Science*, vol. 127, pp. 129-137.

[61] Blois, M. S., 1958. "Antioxidant determinations by the use of a stable free radical." *Nature*, vol. 181, pp. 1199-1200.

[62] Prieto, P., Pineda, M., and Aguilar, M., 1999. "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E." *Analytical Biochemistry*, vol. 269, pp. 337-341.

[63] Gomori, G., 1953. "Human esterases." *The Journal of Laboratory and Clinical Medicine*, vol. 42, pp. 445-453.

[64] Bradford, M. M., 1976. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." *Analytical biochemistry*, vol. 72, pp. 248-254.

[65] Abdelfattah and Lim, J. W., 2021. "Biotechnology application of organic waste management using black soldier fly, Hermetia illucens." *African Journal of Biological Sciences*, vol. 17, pp. 171-187.

[66] Quenea, K., Lamy, I., Winterton, P. B., A., and Dumat, C., 2009. "Interactions between metals and soil organic matter in various particle size fractions of soil contaminated with waste water." *Geoderma*, vol. 149, pp. 217-223.

[67] Khan, M. A., Khan, S., Khan, A., and Alam, M., 2017. "Soil contamination with cadmium, consequences and remediation using organic amendments." *Science of the Total Environment*, vol. 601, pp. 1591-1605.

[68] Ghimire, A., Trably, E., Frunzo, L., Pirozzi, F., Lens, P. N., Esposito, G., and Escudié, R., 2018. "Effect of total solids content on biohydrogen production and lactic acid accumulation during dark fermentation of organic waste biomass." *Bioresource Technology*, vol. 248, pp. 180-186.

[69] Hoornweg, D. and Bhada-Tata, P., 2012. *What a waste: a global review of solid waste management Urban development series;knowledge papers no. 15*. Washington, DC: World Bank.

[70] Christensen, T. H., Scharff, H., and Hjelmar, O., 2011. "Landfilling: Concepts and challenges." *In Solid Waste Technology and Management*, vol. 2, pp. 685-694.

[71] Liu, Huang, B., Bi, X., Ren, Z., Sheng, G., and Fu, J., 2013. "Heavy metals and organic compounds contamination in soil from an e-waste region in South China." *Environmental Science: Processes and Impacts*, vol. 15, pp. 919-929.

[72] Ma, J., Chen, Y., Antoniadis, V., Wang, K., Huang, Y., and Tian, H., 2020. "Assessment of heavy metal (loid) s contamination risk and grain nutritional quality in organic waste-amended soil." *Journal of Hazardous Materials*, vol. 399, p. 123095.

[73] Venegas, A., Rigol, A., and Vidal, M., 2016. "Changes in heavy metal extractability from contaminated soils remediated with organic waste or biochar." *Geoderma*, vol. 279, pp. 132-140.

[74] Azam, I., Afsheen, S., Zia, A., Javed, M., Saeed, R., Sarwar, M. K., and Munir, B., 2015. "Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan." *BioMed Research International*, Available: https://doi.org/10.1155/2015/942751

[75] Rani, M. S., Emmanuel, S., Sreekanth, M. R., and Ignacimuthu, S., 2010. "Evaluation of in vivo antioxidant and hepatoprotective activity of Cassia occidentalis Linn. against paracetamol induced liver toxicity in rats." *Int. J. Pharm. Pharm. Sci.*, vol. 2, pp. 67-70.

[76] Akinrinmade, J. F. and Akinrinde, A. S., 2013. "Effect of oral administration of methanolic extract of Ocimum gratissimum on intestinal ischemia-reperfusion injury in rats." *Eur. J. Med. Plants*, vol. 3, pp. 591-602.

[77] Al-Rubaei, Z. M., Mohammad, T. U., and Ali, L. K., 2014. "Effects of local curcumin on oxidative stress and total antioxidant capacity in vivo study." *Pak. J. Biol. Sci.*, vol. 17, pp. 1237-1241.

[78] Bessa, L. W., Pieterse, E., Marais, J., and Hoffman, L. C., 2020. "Why for feed and not for human consumption? The black soldier fly larvae." *Comprehensive Reviews in Food Science and Food Safety*, vol. 19, pp. 2747-2763.

[79] Diener, S., Zurbrügg, C., and Tockner, K., 2009. "Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates." *Waste Management and Research*, vol. 27, pp. 603-610.

[80] Klammsteiner, T., Turan, V., Fernandez-Delgado, J. M., Oberegger, S., and Insam, H., 2020. "Suitability of black soldier fly frass as soil amendment and implication for organic waste hygienization." *Agronomy*, vol. 10, p. 1578.

[81] Makkar, H. P., Tran, G., Heuzé, V., and Ankers, P., 2014. "State-of-the-art on use of insects as animal feed." *Animal Feed Science and Technology*, vol. 197, pp. 1-33.

[82] Sheppard, D. C., Newton, G. L., Thompson, S. A., and Savage, S., 1994. "A value added manure management system using the black soldier fly." *Bioresource Technology*, vol. 50, pp. 275-279.

[83] Tomberlin, J. K., Adler, P. H., and Myers, H. M., 2009. "Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature." *Environmental Entomology*, vol. 38, pp. 930-934.

[84] Fuso, A., Barbi, S., Macavei, L. I., Luparelli, A. V., Maistrello, L., Montorsi, M., and Caligiani, A., 2021. "Effect of the rearing substrate on total protein and amino acid composition in black soldier fly." *Foods*, vol. 10, p. 1773.

[85] Liu, C., Yao, H., and Wang, C., 2021. "Black soldier fly larvae can effectively degrade oxytetracycline bacterial residue by means of the gut bacterial community." *Frontiers in microbiology*, vol. 12, p. 1565.

[86] Karimi, H., Mahdavi, S., Asgari, L. B., .Moghiseh, E. R., V. D., Minkina, T., and Astatkie, T., 2021. "Insights on the bioremediation technologies for pesticide-contaminated soils." *Environmental Geochemistry and Health*, pp. 1-26. Available: https://doi.org/10.1007/s10653-021-01081-z

[87] Quintella, C. M., Mata, A. M., and Lima, L. C., 2019. "Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils." *Journal of Environmental Management*, vol. 241, pp. 156-166.

[88] Di Marzo, N., Chisci, E., and Giovannoni, R., 2018. "The role of hydrogen peroxide in redox-dependent signaling: homeostatic and pathological responses in mammalian cells." *Cells*, vol. 7, p. 156.

[89] Boopathy, R., 2000. "Factors limiting bioremediation technologies." *Bioresource Technology*, vol. 74, pp. 63-67.

[90] Riber, C., Petersen, C., and Christensen, T. H., 2009. "Chemical composition of material fractions in Danish household waste." *Waste Management*, vol. 29, pp. 1251-1257.

[91] Wuana, R. A. and Okieimen, F. E., 2011. "Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation." p. 20. Available: http://doi.org/10.5402/2011/402647

[92] Van Dooren, C., Janmaat, O., Snoek, J., and Schrijnen, M., 2019. "Measuring food waste in Dutch households: A synthesis of three studies." *Waste management*, vol. 94, pp. 153-164.

[93] Gasco, L., Biancarosa, I., and Liland, N. S., 2020. "From waste to feed: A review of recent knowledge on insects as producers of protein and fat for animal feeds." *Current Opinion in Green and Sustainable Chemistry*, vol. 23, pp. 67-79.

[94] Zaman, K., MacGill, R. S., Johnson, J. E., Ahmad, S., and Pardini, R. S., 1995. "An insect model for assessing oxidative stress related to arsenic toxicity." *Archives of Insect Biochemistry and Physiology*, vol. 29, pp. 199-209.

[95] Farahat, A. A., Al-Sayed, A. A., and Mahfoud, N. A., 2010. "Compost and other organic and inorganic fertilizers in the scope of the root-knot nematode reproduction and control." *Egyptian J. Agronematol*, vol. 9, pp. 18-29.

[96] Yousef, H. A., Abdelfattah, E. A., and Augustyniak, M., 2017. "Evaluation of oxidative stress biomarkers in Aiolopus thalassinus (Orthoptera: Acrididae) collected from areas polluted by the fertilizer industry." *Ecotoxicology*, vol. 26, pp. 340-350.

[97] Fang, Y. Z., Yang, S., and Wu, G., 2002. "Free radicals, antioxidants, and nutrition." *Nutri.*, vol. 18, pp. 872-879.

[98] Gaur, N., Flora, G., Yadav, M., and Tiwari, A., 2014. "A review with recent advancements on bioremediation-based abolition of heavy metals." *Environmental Science: Processes and Impacts*, vol. 16, pp. 180-193.

[99] Shukla, A. K., Pragya, P., and Chowdhuri, D. K., 2011. "A modified alkaline Comet assay for in vivo detection of oxidative DNA damage in Drosophila melanogaster." *Mut Rese/Gene Toxicol. Environ. Mutat.*, vol. 726, pp. 222-226.

[100] Tuncsoy, B. S., Tuncsoy, M., Gomes, T., Sousa, V., Teixeira, M. R., Bebianno, M. J., and Ozalp, P., 2019. "Effects of copper oxide nanoparticles on tissue accumulation and antioxidant enzymes of Galleria mellonella L." *Bulletin of Environmental Contamination and Toxicology*, vol. 102, pp. 341-346.

[101] Baryla, A., Laborde, C., Montillet, J. L., Triantaphylides, C., and Chagvardieff, P., 2000. "Evaluation of lipid peroxidation as a toxicity bioassay for plants exposed to copper." *Environmental Pollution*, vol. 109, pp. 131-135.

[102] Lushchak, V. I., 2011. "Environmentally induced oxidative stress in aquatic animals." *Aquat Toxicol.*, vol. 101, pp. 13-30.

[103] Hermes-Lima, M., 2004. "Oxygen in biology and biochemistry: role of free radicals." *Funct. Meta.: Regulation and adaptation*, vol. 1, pp. 319-966.

[104] Korsloot, A., Van Gestel, C. A., and Van Straalen, N. M., 2004. *Environmental stress and cellular response in arthropods*. CRC Press.

[105] Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O., 2012. "Oxidative stress and antioxidant defense." *The World Allergy Organ J.*, vol. 5, pp. 9-19.

[106] Halliwell, B. and Gutteridge, J. M., 2015. *Free radical bio med*. USA: Oxford University Press.
[107] Brattsten, L. B., 1988. "Enzymic adaptations in leaf-feeding insects to host-plant allelochemicals." *Journal of Chemical Ecology*, vol. 14, pp. 1919-1939.
[108] Fischer, H. and Romano, N., 2021. "Fruit, vegetable, and starch mixtures on the nutritional quality of black soldier fly (Hermetia illucens) larvae and resulting frass." *Journal of Insects as Food and Feed*, vol. 7, pp. 319-327.
[109] Anspaugh, D. D., Rose, R. L., Koehler, P. G., Hodgson, E., and Roe, R. M., 1994. "Multiple mechanisms of pyrethroid resistance in the German cockroach, Blattella germanica (L.)." *Pesticide Biochemistry and Physiology*, vol. 50, pp. 138-148.
[110] Sahgal, A., Kumar, S., and Pillai, M. K. K., 1994. "Microplate assay of elevated esterase activity in individual pyrethroid-resistant mosquitoes." *Journal of Biosciences*, vol. 19, pp. 193-199.
[111] Ishaaya, I. and Casida, J. E., 1980. "Properties and toxicological significance of esterases hydrolyzing permethrin and cypermethrin in Trichoplusia ni larval gut and integument." *Pesticide Biochemistry and Physiology*, vol. 14, pp. 178-184.
[112] Mishra, K., Ojha, H., and Chaudhury, N. K., 2012. "Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results." *Food Chemistry*, vol. 130, pp. 1036-1043.
[113] Davalos, A., Miguel, M., Bartolome, B., and Lopez-Fandino, R., 2004. "Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis." *J. Food Prot.*, vol. 67, pp. 1939–1944.
[114] Oakeshott, J. G., Claudianos, C., Campbell, P. M., Newcomb, R. D., and Russell, R. J., 2005. *Biochemical genetics and genomics of insect esterases. In: Gilbert, L.I., Latrou, K., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science-Pharmacology*. Oxford: Elsevier. p. 309e381.
[115] Chen, C., Liu, Y., Shi, X., Desneux, N., Han, P., and Gao, X., 2017. "Elevated carboxylesterase activity contributes to the lambda-cyhalothrin insensitivity in quercetin fed Helicoverpa armigera (Hübner)." *PLoS One*, vol. 12, p. e0183111.
[116] Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z., and Nagy, G., 2007. "Antioxidant measurements." *Physiological Measurement*, vol. 28, p. R41.
[117] Ghiselli, A., Serafini, M., Natella, F., and Scaccini, C., 2000. "Total antioxidant capacity as a tool to assess redox status: critical view and experimental data." *Free Radical Biology and Medicine*, vol. 29, pp. 1106-1114.
[118] Meng, J. Y., Zhang, C. Y., Zhu, F., Wang, X. P., and Lei, C. L., 2009. "Ultraviolet light-induced oxidative stress: effects on antioxidant response of Helicoverpa armigera adults." *Journal of Insect Physiology*, vol. 55, pp. 588-592.
[119] Caimi, C., Gasco, L., Biasato, I., Malfatto, V., Varello, K., Prearo, M., and Gai, F., 2020. "Could dietary black soldier fly meal inclusion affect the liver and intestinal histological traits and the oxidative stress biomarkers of Siberian sturgeon (Acipenser baerii) juveniles?" *Animals*, vol. 10, p. 155.
[120] Leskovec, J., Levart, A., Nemec, Svete, A., Perić, L., Dukić, Stojčić, M., and Žikić, D., 2018. "Effects of supplementation with α-tocopherol, ascorbic acid, selenium, or their combination in linseed oil-enriched diets on the oxidative status in broilers." *Poultry science*, vol. 97, pp. 1641-1650.
[121] Tripathi, B. N., Mehta, S. K., Amar, A., and Gaur, J. P., 2006. "Oxidative stress in Scenedesmus sp. during short-and long-term exposure to Cu2+ and Zn2+." *Chemosphere*, vol. 62, pp. 538-544.
[122] Kaur, G., Athar, M., and Alam, M. S., 2008. "Quercus infectoria galls possess antioxidant activity and abrogates oxidative stress-induced functional alterations in murine macrophages." *Chemico-Biological Interactions*, vol. 171, pp. 272-282.
[123] Oghenesuvwe, E. E. and Paul, C., 2019. "Edible insects bio-actives as anti-oxidants: Current status and perspectives." *Journal of Complementary Medicine*, vol. 10, pp. 89-102.
[124] Hilliker, A. J., Duyf, B., Evans, D., and Phillips, J. P., 1992. "Urate-null rosy mutants of Drosophila melanogaster are hypersensitive to oxygen stress." *Proceedings of the National Academy of Sciences*, vol. 89, pp. 4343-4347.
[125] Suh, H. J. and Kang, S. C., 2012. "Antioxidant activity of aqueous methanol extracts of Protaetia brevitarsis Lewis (Coleoptera: Scarabaedia) at different growth stages." *Natural Product Research*, vol. 26, pp. 510-517.
[126] Suh, H. J., Kim, S. R., Hwang, J. S., Kim, M. J., and Kim, I., 2011. "Antioxidant activity of aqueous methanol extracts from the lucanid beetle, Serrognathus platymelus castanicolor Motschulsky (Coleoptera: Lucanidae)." *Journal of Asia-Pacific Entomology*, vol. 14, pp. 95-98.
[127] Fawole, F., Adeoye, A., Tiamiyu, L., Ajala, K., Obadara, S., and Ganiyu, I., 2020. "Substituting fishmeal with Hermetia illucens in the diets of African catfish (Clarias gariepinus): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker." *Aquaculture*, vol. 518, p. 734849.
[128] Surendra, K. C., Tomberlin, J. K., van Huis, A., Cammack, J. A., Heckmann, L. H. L., and Khanal, S. K., 2020. "Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.))(Diptera: Stratiomyidae)(BSF)." *Waste Management*, vol. 177, pp. 58-80.
[129] Nguyen, T. T., Tomberlin, J. K., and Vanlaerhoven, S., 2015. "Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste." *Environmental Entomology*, vol. 44, pp. 406-410.
[130] Lock, E. R., Arsiwalla, T., and Waagbø, R., 2016. "Insect larvae meal as an alternative source of nutrients in the diet of A tlantic salmon (S almo salar) postsmolt." *Aquaculture Nutrition*, vol. 22, pp. 1202-1213.

[131] De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., *et al.*, 2015. "Nutritional value of two insect larval meals (Tenebrio molitor and Hermetia illucens) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy." *Anim. Feed Sci. Technol.*, vol. 209, pp. 211–218.

[132] Tschirner, M. and Simon, A., 2015. "Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed." *J. Insects Food Feed*, vol. 1, pp. 1–12.

[133] Verkerk, M. C., Tramper, J., Van Trijp, J. C. M., and Martens, D. E., 2007. "Insect cells for human food." *Biotechnol. Adv.*, vol. 25, pp. 198–202.

[134] Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., and Vantomme, P., 2013. *Edible insects: Future prospects for food and feed security*. Rome: Italy: Food and Agriculture Organization of the United Nations.

[135] Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., *et al.*, 2017. "Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates." *J. Sci. Food Agric.*, vol. 97, pp. 2594–2600.

[136] Tran, G., Heuzé, V., and Makkar, H. P. S., 2015. "Insects in fish diets." *Anim. Front*, vol. 5, pp. 37–44.

[137] Zhang, J., Shi, Z., Gao, Z., Wen, Y., Wang, W., Liu, W., and Zhu, F., 2021. "Identification of three metallothioneins in the black soldier fly and their functions in Cd accumulation and detoxification." *Environmental Pollution*, vol. 286, p. 117146.

[138] Diener, S., Solano, N. M. S., Gutiérrez, F. R., Zurbrügg, C., and Tockner, K., 2011. "Biological treatment of municipal organic waste using black soldier fly larvae." *Waste and Biomass Valorization*, vol. 2, pp. 357-363.

[139] Kavitha, P. and Rao, J. V., 2007. "Oxidative stress and locomotor behaviour response as biomarkers for assessing recovery status of mosquito fish, Gambusia affinis after lethal effect of an organophosphate pesticide, monocrotophos." *Pesticide Biochemistry and Physiology*, vol. 87, pp. 182-188.

[140] Liu, L. and Xia, Q., 2016. "Research progresses about the effect of copper pollution on the growth and reproduction of insects." *Journal of Environmental Entomology*, vol. 38, pp. 451-456.

[141] Surendra, K. C., Tomberlin, J. K., van Huis, A., Cammack, J. A., Heckmann, L. H. L., and Khanal, S. K., 2020. "Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.))(Diptera: Stratiomyidae)(BSF)." *Waste Management*, vol. 117, pp. 58-80.