BRAZILIAN JOURNAL OF BIOLOGY®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Analysis of antioxidants in water striders (Hemiptera: Gerridae) as bioindicator of water pollution

Análise de antioxidantes em water striders (Hemiptera: Gerridae) como bioindicador de poluição da água

R. Saleem[a,b] and S. Afsheen[a*]

[a]University of Gujrat, Department of Zoology, Hafiz Hayat Campus, Gujrat, Punjab, Pakistan
[b]University of Sialkot, Sialkot, Punjab, Pakistan

Abstract

The antioxidant enzyme system is an important defense mechanism to cope with Reactive Oxygen Species (ROS) produced due to exposure to heavy metals. In the present study lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd) and nickel (Ni) in water and the antioxidant activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) was investigated in three species (*Metrocoris communis*, *Limnogonus fossarum fossarum,* and *Aquarius adelaidis*) of water striders collected from the industrial triangle of Punjab, Pakistan. The results of present study revealed that Pb, Cr, As, Cd and Ni were according to the permissible amount of WHO. The antioxidant activity of SOD, CAT and POD was found significantly different among species against oxidative stress, but found the highest activity of determining parameters in *A. adelaidis*. This is one of the pioneer studies in Pakistan reporting the role of water striders as a bioindicator of heavy metals present in the water through antioxidants enzyme variations. The current results supported that variant level of antioxidant enzyme activities in different species of water strider were reflective of heavy metal pollution in the Industrial triangle of Punjab, Pakistan and will be a useful ecotoxicological tools to evaluate the detrimental effects of heavy metal pollutants in aquatic organisms.

Keywords: antioxidant enzyme, water strider, industrial triangle, heavy metals, pollution.

Resumo

O sistema enzimático antioxidante é um importante mecanismo de defesa para lidar com Espécies Reativas de Oxigênio (EROs) produzidas por causa da exposição a metais pesados. No presente estudo, o chumbo (Pb), o cromo (Cr), o arsênio (As), o cádmio (Cd), o níquel (Ni) em água e a atividade antioxidante da superóxido dismutase (SOD), catalase (CAT) e peroxidase (POD) foram investigados em três espécies (*Metrocoris communis*, *Limnogonus fossarum fossarum* e *Aquarius adelaidis*) de gafanhotos coletados no triângulo industrial de Punjab, Paquistão. Os resultados do presente estudo revelaram que Pb, Cr, As, Cd e Ni estavam de acordo com a quantidade permitida pela OMS. A atividade antioxidante de SOD, CAT e POD foi significativamente diferente entre as espécies diante do estresse oxidativo, mas encontrou a maior atividade de determinação de parâmetros em *A. adelaidis*. Este é um dos estudos pioneiros no Paquistão que relatam o papel dos water striders como bioindicador de metais pesados presentes na água por meio de variações de enzimas antioxidantes. Os resultados atuais indicaram que o nível variante de atividades de enzimas antioxidantes em diferentes espécies de water striders refletiu a poluição por metais pesados no triângulo industrial de Punjab, Paquistão, e será uma ferramenta ecotoxicológica útil para avaliar os efeitos prejudiciais de poluentes de metais pesados em organismos aquáticos .

Palavras-chave: enzima antioxidante, water strider, triângulo industrial, metais pesados, poluição.

1. Introduction

Various metallic elements like arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) act as the major environmental pollutants in the water due to urbanization and industrialization (Rehman et al., 2008). The presence of these heavy metals is detrimental for the aquatic ecosystem and biodiversity due to bioaccumulation of heavy metals through food chain in the body (Aljahdali & Alhassan, 2020; Govind & Madhuri, 2014). Bioaccumulation of these trace metal elements induces the production of reactive oxygen species (ROS), which might cause oxidative stress in the organisms exposed to these toxic metals (Cuny et al., 2004). In addition, biotic and abiotic factors such as pathogens, drought, salinity, chilling, UV-B radiation, toxic substances, and diet produce oxidative stress in all aerobes. Oxidative stress is harmful to organisms

*e-mail: sumera.afsheen@uog.edu.pk
Received: November 10, 2021 – Accepted: February 8, 2022

disrupting the structure of lipids, proteins, free amino acids and nucleic acids (Cross et al., 1987; Missirlis et al., 2001; Schieber and Chandel, 2014; Sharma et al., 2012). Organisms may avoid environmental stress by adapting behaviorally, biochemically and through other physiological mechanisms (Migula et al., 2004).

ROS produces highly reactive chemical species like free oxygen, hydroxyl radical and hydrogen peroxide that can initiate the harmful chain reactions causing damage to cells. ROS scavengers act as a secondary messenger or signaling molecule to control physiological activities against environmental stress by balancing ROS production (Finkel, 2011; Sharma et al., 2012).

Insects possess antioxidant enzyme systems such as SOD, CAT, POD, glutathione transferase, and glutathione reductase to detoxify ROS (Ahmad, 1992). Similarly, some water-soluble and lipid-soluble antioxidants such as ascorbate, glutathione, tocopherols and carotenoids have not been well studied in insects. Still, may play significant antioxidant roles to overcome ROS toxicity (Felton and Summers, 1995).

Water striders are predators that feed on terrestrial insects that fall in the water and planktonic crustaceans and fish larvae (Cheng, 1974). It is reported that water striders can accumulate heavy metals due to the scavenging activities and acts as a bioindicator to heavy metal contamination (Jardine et al., 2005; Nummelin et al., 1998). The present study was designed to study the antioxidant activity in water striders (Hemiptera: Gerridae) to assess of their role as bioindicators. These insects are very common, widely distributed in small streams to large rivers, ponds, lakes, and even over the ocean's surface (Andersen, 1982; Andersen, 1995; Spence and Anderson, 1994). However, so far, no published literature is available on antioxidants produced to cope with heavy metal stress in water striders. In this study, we evaluated the antioxidant enzyme activity of SOD, CAT and POD in commonly available species of water striders collected from the industrial triangle of Punjab, Pakistan containing heavy metals in the water bodies.

2. Materials and Methods

2.1. Study areas

The industrial triangle comprises three districts, i.e. Gujrat (32.39°–33.03° N, 73.59°–74.46° E), Sialkot (32.05°–32.84° N, 74.20°–74.94° E) and Gujranwala (31.81°–32.55° N, 73.67°–74.58°E) districts of Punjab, Pakistan. This triangle is a business bay for agriculture, ceramics, fan, furniture, textile, sports, steel and leather. These industries release waste water effluents in the water bodies, which is the leading cause of water pollution (Azam et al., 2015; Nasir et al., 2012; Rafique et al., 2010). In addition, metal elements including arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) have been reported in water due to discharge of municipal and industrial waste in these cities (Ali et al., 2013; Masood et al., 2019; Qureshi et al., 2015; Waseem et al., 2014).

2.2. Water striders collection

Adult water striders were collected from 23 sites of Industrial Triangle of Punjab viz district Sialkot: Marala Ravi Link Canal near Basiwala Pul (S1), Marala Ravi Link Canal near Kotli Baba Faqir Chand (S2), Marala Ravi Link Canal near Canal City Housing Society (S3), River Chenab Head Marala (S4), Upper Chenab Canal near Malkhnawala (S5), Upper Chenab Canal near Zafarwali Bus Stop (S6), Marala Ravi Link Canal near Motra (S7), Banbawali Ravi Badian Canal near Circular Road, Daska (S8), district Gujrat: Upper Jhelum Canal near Pathan Colony Kharian (S9), Upper Jhelum Canal near village Jaura Kharian (S10), Upper Jhelum Canal Grand Trunk Road Sarai Alamgir (S11), Upper Jhelum Canal near Simbli Sharif Road Sarai Alamgir (S12), River Jhelum (S13), Upper Jhelum Canal Gujrat (S14), Halsi Nala Gujrat (S15), district Gujranwala: Upper Chenab Canal, Nandipur (S16), Upper Chenab Canal near Chan Da Qilla (S17), Upper Chenab Canal, Kamoki-Tatle Aali Road (S18), Nullah near National High Way Kamoki (S19), Nowshera Virkan (S20), Nullah Palkhu, Grand Trunk Road Wazirabad (S21), Gammon Lake, Wazirabad (S22), and Lower Chenab Canal Wazirabad (S23) (See Figures 1, 2, 3, 4) during October and November 2018. Geographical and Ecological data are shown in Table 1.

2.3. Enzyme assay

Three species of water striders were selected for the assay. Adult gerrids were brought alive to the laboratory to further analyze CAT, POD and SOD. Water striders acclimatized in the laboratory were used as control. Fresh insect samples were anesthetized using ice with their wings and legs excised to check the enzyme activity. Samples were homogenized at 4°C in 2.5 ml of 0.05 M Sodium/Potassium buffer at pH 7.4. The homogenates were filtered and centrifuged for 10 min at 15,000g and stored at -70ºC for further analysis (Lee et al., 2005).

The SOD activity was determined using a method by (Kakkar et al., 1984) with some modifications of (Das et al., 2000). The absorbance was measured at 560nm. Catalase activity was assayed following Luck (1974). CAT activity was determined by measuring the absorbance at 240nm. POD assay was carried out following the method of Pütter (1974) by measuring the absorbance at 470nm.

2.4. Water samples collection and heavy metal analysis

Water samples were also collected from the same sites in sterilized plastic bottles washed with double distilled water and 20% HNO_3 and brought to the laboratory. Water samples were filtered using a 0.45-mm Whatman pore membrane. Water samples were acidified with nitric acid (HNO_3, 69%) to prevent adsorption and crystallization. Water samples were stored in a refrigerator at 4°C until analysis. The water samples were analyzed for Pb, Cr, As, Cd and Ni using Flame Atomic Absorption Spectrophotometer (APHA, 2005).

2.5. Statistical analysis

The sample sites are mentioned on the map using the ArcMap 10.3.1 version. For enzyme assay, the three variables were analyzed by one-way Analysis of Variance (ANOVA)

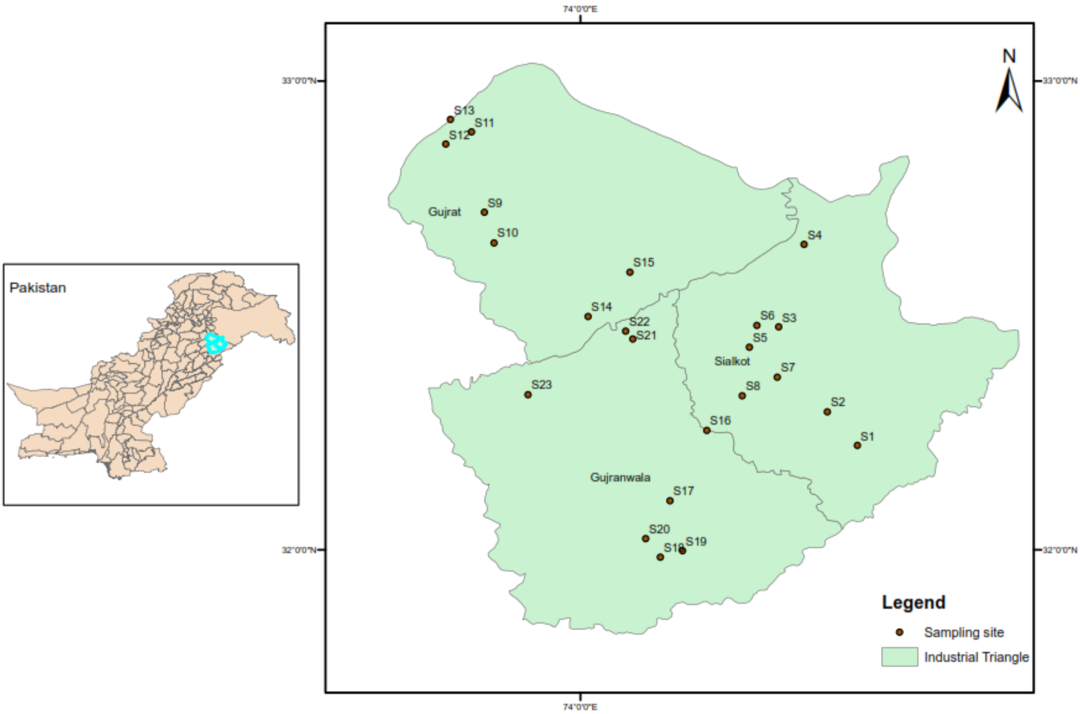

Figure 1. Map of sampling sites showing industrial triangle of Punjab, Pakistan using ArcMap 10.3.1 version.

Figure 2. Sampling site at Upper Jhelum Canal, Grand Trunk Road, Sarai Alamgir, district Gujrat.

Figure 3. Sampling site at Gammon lake, Wazirabad, district Gujranwala.

using the software 'R'version 3.5.3 (R Core Team, 2017). To check the distribution of the reads among the samples, bar charts were plotted using R-package"ggplot2"(Wickham, 2009). The normality of the data was checked by the Shapiro-Wilk test in 'R'. After analyzing the data using one way ANOVA, it was observed that there was a significant difference between species based on three considered locations. Therefore, the data were analyzed using the POSTHOC test to determine the significant difference of *M. communis* (sp1) with *F. fossarum* (sp2) and *A. adeladis* (sp3) and vice versa. Similarly, the POSTHOC test was also used to analyze the significant difference of enzyme activity in species between sites in package DescTools available in R software version 3.5.3. Descriptive statistics and correlation analysis were used for heavy metals in 'R'. A bar chart was created to show average values of heavy metals using package "ggplot2" (Wickham, 2009).

3. Results

The SOD, CAT and POD were calculated to determine the enzymatic activity in considered species (*M. communis, L.*

Figure 4. Sampling site at Marala Ravi Link Canal near Basiwala Pul, district Sialkot.

fossarum and A. adelaidis) collected from water containing heavy metals. Statistical analysis was performed to explore the significant difference among species ($p \leq 0.05$) based on three considered sites (Gujranwala, Gujrat and Sialkot). Graphical visualization of SOD, POD and CAT is represented in Figure 5.

Graphical representation of SOD showed that SOD activity was highest in sp 2 (*L. fossarum)* in Gujrat. SOD activity was high in sp 3 (*A. adelaidis*) in Gujranwala and Sialkot than sp 1 (*M. communis*) (See Figure 5a). One way ANOVA revealed that enzyme activity in three considered species was significantly different. Similarly, according to three considered sites, it was observed that the SOD activity was also significantly different.

The POSTHOC test was also performed to observe the difference of SOD activity among sp 1, sp 2 and sp 3; and vice versa. It was determined that the enzymatic activity was significantly different ($p \leq 0.05$) in sp1, sp2 and sp3. Furthermore, by considering sites it was observed that there was a significant difference ($p \leq 0.05$) between Gujrat-Gujranwala and Sialkot-Gujrat but the results were distinct within Sialkot-Gujranwala (See Figure 6a).

It was illustrated in Figure 5b. that CAT activity was higher in *A. adelaidis* (sp 3) than *L. fossarum* (sp2) and *M. communis* (sp1). One way ANOVA showed that CAT activity was significantly different in three species. Similarly, CAT activity was significantly different based on three sites.

The POSTHOC test determined the difference in CAT activity among the three species. CAT activity was significantly different in the three species. According to the sites, CAT activity was significantly different in Gujrat-Gujranwala and Sialkot-Gujranwala but in Sialkot-Gujrat CAT activity was non-significant ($p > 0.05$) (See Figure 6b).

The POD activity was highest in *A. adelaidis* (sp 3) in three different sites (See Figure 5c). The POD activity in *L. fossarum* (sp 2) was higher in Gujrat than Gujranwala and Sialkot where *M. communis* (sp1) showed the lowest activity. One way ANOVA showed that the POD activity was significantly different in three considered species ($P < 0.05$). Similar significant results were determined based on three considered sites (See Figure 6c).

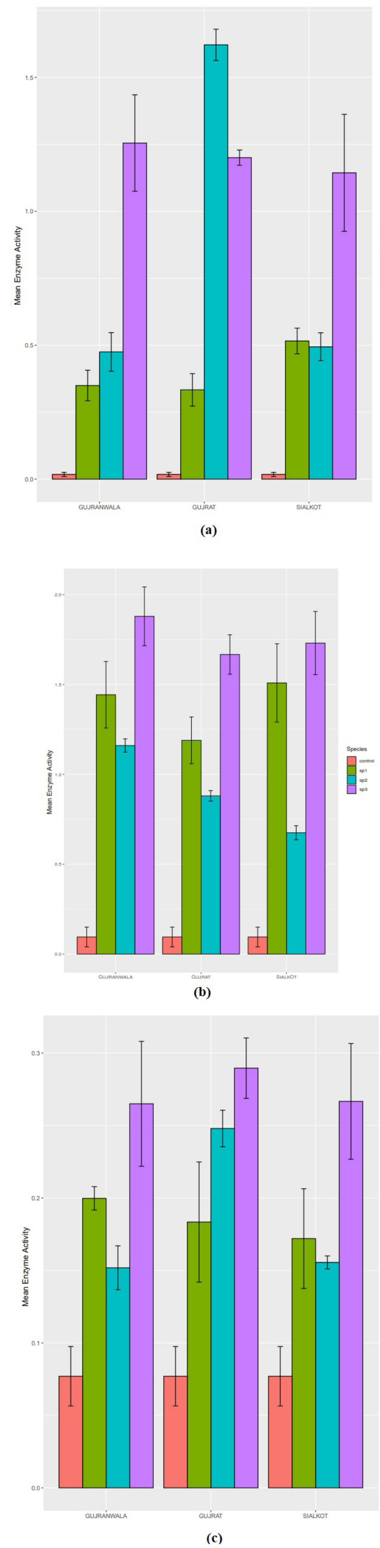

Figure 5. (a) Activity of Superoxidase Dismutase in different species of water strider from three districts. (b) Activity of Catalase in different species of water strider from three districts. (c) Activity of Peroxidase in different species of water strider from three districts. **sp1=** *M. communis*, **sp2**= *L. fossarum*, **sp3=** *A. adelaidis*.

Descriptive statistics of heavy metals in water samples collected from three Districts presented in Table 2. The results revealed that the mean value of lead in Sialkot, Gujranwala and Gujrat was 0.012 ± 0.001, 0.013 ± 0.004 and 0.012 ± 0.004, respectively. Similarly, the amount of Chromium was less than water quality standards by WHO in Sialkot, Gujranwala and Gujrat, the average contents were 0.006 ± 0.001, 0.006 ± 0.001 and 0.005 ± 0.001 respectively. The average amount of Arsenic in district Sialkot, Gujranwala and Gujrat was 0.003 ± 0.001, 0.003 ± 0.001 and 0.003 ± 0.001which was within the limit of standard value of WHO (0.01mg/l). The mean content of cadmium was 0.002 ± 0.001, 0.006 ± 0.001 and 0.004 ± 0.001 in Sialkot, Gujranwala and Gujrat respectively. On the other hand, the average amount of nickel was high in district Gujrat than Sialkot and Gujranwala, i.e. 0 .068 ± 0.013 (See Table 2, Figure 7).

Correlation analysis of heavy metals of three districts displayed in Table 3. Lead showed a positive association with chromium (r = 0.009) and arsenic (r = 0.113) but a negative relationship with cadmium (r = -0.064) and nickel (r = -0.269). A negative relationship of chromium was revealed with nickel (r = -0.065) but showed a positive relationship with arsenic (r = 0.558) and cadmium (r = 0.005). Similarly, a positive association was found between arsenic and cadmium (r = 0.026) but a negatively correlated with nickel (r = -0.141). The study determined a negative relationship between cadmium and nickel among all considered districts.

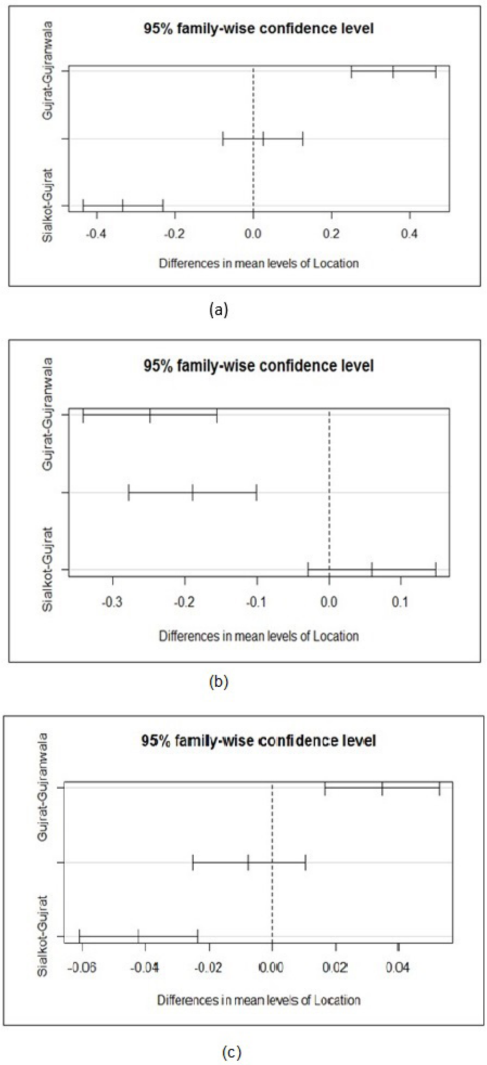

Figure 6. (a) Superoxidase Dismutase in water striders collected from Gujrat, Gujranwala and Sialkot using POSTHOC test; (b) Catalase in water striders collected from Gujrat, Gujranwala and Sialkot using POSTHOC test; (c) Peroxidase in water striders collected from Gujrat, Gujranwala and Sialkot by using POSTHOC test.

4. Discussion

In the current study, heavy metals in water samples were determined and evaluated the antioxidant activity in water striders collected from water bodies. It was found that lead, chromium and arsenic were in the range of permissible amounts compared with Pakistan National

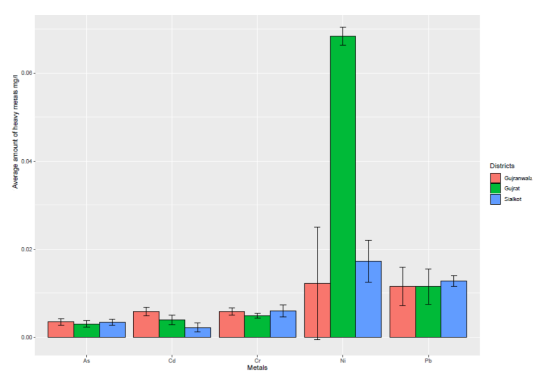

Figure 7. Average amount of heavy metals in water samples collected from industrial triangle of Punjab.

Table 1. Geographical location and Ecological data of Sampling Sites.

District	Latitude	Longitude	Average Air Temperature (°C)	Average Water Temperature (°C)	Average Humidity (%)
Sialkot	32.05°–32.84° N	74.20°–74.94° E	23.12 ± 2.9	19.75± 0.81	35 ± 6.06
Gujranwala	31.81°–32.55° N	73.67°–74.58°E	22.87 ± 2.8	19.37± 1.50	43.12 ± 4.11
Gujrat	32.39°–33.03° N	73.59°–74.46° E	29.42 ± 1.8	23.28 ± 0.70	37.85 ± 9.76

Table 2. Descriptive statistics for heavy metals of water samples in Industrial Triangle.

Districts	Metals (mg/l)	N	Range	Minimum	Maximum	Mean		Std. Deviation	Skewness		Kurtosis	
		Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic	Std. Error	Statistic	Std. Error
Sialkot	Pb	8	0.009	0.008	0.017	0.012	0.001	0.004	-.181	.752	-1.481	1.481
	Cr	8	0.013	0.001	0.014	0.006	0.001	0.004	1.243	.752	2.263	1.481
	As	8	0.006	0.002	0.008	0.003	0.001	0.002	2.113	.752	5.007	1.481
	Cd	8	0.007	0.000	0.007	0.002	0.001	0.003	1.268	.752	-.222	1.481
	Ni	8	0.043	0.000	0.043	0.017	0.005	0.014	0.685	.752	.705	1.481
Gujranwala	Pb	8	0.027	0.004	0.030	0.013	0.004	0.011	0.770	.752	-1.577	1.481
	Cr	8	0.005	0.004	0.009	0.006	0.001	0.002	0.791	.752	.266	1.481
	As	8	0.005	0.006	0.007	0.003	0.001	0.002	0.916	.752	-.969	1.481
	Cd	8	0.008	0.003	0.01	0.006	0.001	0.003	0.372	.752	-1.924	1.481
	Ni	8	0.018	0.000	0.017	0.012	0.002	0.006	-1.425	.752	2.295	1.481
Gujrat	Pb	7	0.026	0.004	0.029	0.012	0.004	0.012	1.201	.794	-.793	1.587
	Cr	7	0.006	0.003	0.009	0.005	0.001	0.002	.495	.794	-.885	1.587
	As	7	0.005	0.001	0.006	0.003	0.001	0.002	.585	.794	-1.852	1.587
	Cd	7	0.007	0.002	0.008	0.004	0.001	0.002	.880	.794	-.410	1.587
	Ni	7	0.079	0.030	0.108	0.068	0.013	0.033	-.103	.794	-2.375	1.587

Table 3. Correlation for heavy metals in Industrial Triangle of Punjab.

Metals (mg/l)	Pb	Cr	As	Cd	Ni
Pb	1	0.009	0.113	-0.064	-0.269
Cr		1	0.558**	0.005	-0.065
As			1	0.026	-0.141
Cd				1	-0.044
Ni					1

**Correlation is significant at the 0.01 level (2-tailed).

Environmental Quality Standards (NEQS, 2000) and World Health Organization (WHO, 2011). In contrast, it was found the amount of lead and chromium was high in drinking water in Sialkot (Ullah et al., 2009). In contrast, Masood et al. (2019) assessed the heavy metals in the drinking water of the industrial zone of Gujrat and found high level than the permissible amount. Similarly, Waseem et al. (2014) reported high heavy metal concentrations in water, soil and vegetables in Pakistan. The presence of heavy metals in the study area was due to the discharge of industrial effluents, anthropogenic activities, agrochemical discharge, domestic sewage or the effect of runoff that ends up in water sources. Cuny et al. (2004) reported that these heavy metals accelerate the ROS activity in insects which cause oxidative stress. It has been well documented that high oxidative stress leads to the production of SOD, CAT and POD (Dutta et al., 2016). In order to find the differences in the antioxidant activity of SOD, CAT and POD, three species of water strider were assessed, collected from three districts of the Industrial triangle of Punjab. SOD, CAT and POD activity was significantly different in three species of water strider collected from study area showing the high concentration of heavy metals. SOD, CAT and POD are important enzymes that can work against oxidative stress produced by ROS activities in the cell (Yang et al., 2010). It has been evaluated that SOD is involved in removing superoxide radicals (Celino et al., 2011). SOD activity was found in three species of water strider but it was observed that the catalytic activity of SOD was high in *A. adelaidis* in district Sialkot and Gujranwala but *L. fossarum* showed more activity in district Gujrat.

It was also reported that SOD activity was high due to metal exposure in *Oxya hyla hyla* (Azam et al., 2017). Similarly, the SOD activity was high in the brain of 5th instar nymphs of *Aiolopus thalassinus,* collected from high polluted site (Yousef et al., 2019). In contrast, SOD activities were reduced in carnivorous *Pterostichus oblongopunctatus* collected from a highly polluted site than a less polluted site

(Migula et al., 2004). There was a relationship between CAT and SOD because this enzyme catalyzes the reduction of H_2O_2 and lipid hydroperoxides (Felton and Summers, 1995). It is well documented that CAT works efficiently when the cellular concentration of H_2O_2 is high (Ahmad et al., 1991). CAT activity was significantly elevated in three species of water striders. Similar findings were found in the 3rd and 5th instars of armyworm when exposed to peroxidant allelochemicals (Pritsos et al., 1988). Barata et al. (2005) also reported high catalytic activity of CAT in Caddisfly larvae of *Hydropsyche exocellata* collected from metal-polluted water. Similarly, (Islam et al., 2019) revealed that muga silkworm, *Antheraea assamensis* showed high CAT activity when exposed to heavy metals. POD is present in many insects and removes H_2O_2 during oxidative stress (Felton & Summers, 1995). In the current study, three species of water strider showed POD activity collected from Industrial waste water. Our results are in line with previous studies that also reported high POD activity in *O. hyla hyla* when exposed to Cd, Pb and Hg (Azam et al., 2017). Similarly, Wu & Yi (2015) found that *Galleria mellonella* larvae showed high POD activity when exposed to dietary Pb and Cr. Similarly, POD was investigated in the midgut of larval *Helicoverpa zea* due to exposure to xenobiotics. (Felton and Summers, 1995). The antioxidant enzymes activities in water striders might an attempt to neutralize the detrimental effect of the ROS on heavy metal exposure.

5. Conclusion

Our results indicate that metal pollutants induced oxidative stress in three species of water strider. SOD, CAT and POD activities were significantly different in three species of water strider and provide a protective mechanism against the damaging effects of reactive oxygen species in water striders. Changes in the antioxidant enzyme activity in three species of water strider validate their role as a bioindicator and biomarker of heavy metals which are significant environmental pollutants. The present study is the pioneering work on the water strider of Pakistan, which will lead to further investigation of the antioxidant activity in water strider using different parameters in the laboratory.

Acknowledgements

The authors thank late Ms. Sonia Iqbal (Lecturer, Department of Statistics, University of Sialkot, Sialkot, Pakistan) for her assistance with statistical analysis whenever required.

Abbreviations

SOD, superoxide dismutase; POD, Peroxidase; CAT, Catalase; OH, hydroxyl radical, ROS, Reactive Oxygen Species; sp, species; WHO, World Health Organization; NEQS, National Environmental Quality Standards.

References

AHMAD, S., 1992. Biochemical defence of pro-oxidant plant allelochemicals by herbivorous insects. *Biochemical Systematics and Ecology*, vol. 20, no. 4, pp. 269-296. http://dx.doi.org/10.1016/0305-1978(92)90040-K.

AHMAD, S., DUVAL, D.L., WEINHOLD, L.C. and PARDINI, R.S., 1991. Cabbage looper antioxidant enzymes: tissue specificity. *Insect Biochemistry*, vol. 21, no. 5, pp. 563-572. http://dx.doi.org/10.1016/0020-1790(91)90111-Q.

ALI, Z., MALIK, R.N. and QADIR, A., 2013. Heavy metals distribution and risk assessment in soils affected by tannery effluents. *Chemistry and Ecology*, vol. 29, no. 8, pp. 676-692. http://dx.doi.org/10.1080/02757540.2013.810728.

ALJAHDALI, M.O. and ALHASSAN, A.B., 2020. Metallic pollution and the use of antioxidant enzymes as biomarkers in *Bellamya unicolor* (Olivier, 1804) (Gastropoda: bellamyinae). *Water (Switzerland)*, vol. 12, no. 1, pp. 202. http://dx.doi.org/10.3390/w12010202.

AMERICAN PUBLIC HEALTH ASSOCIATION – APHA, 2005. *Standard methods for the examination of water and wastewater*. 21st ed. Washington: American Public Health Association/American Water Works Association/Water Environment Federation.

ANDERSEN, N.M., 1982. The semiaquatic bugs (Hemiptera, Gerromorpha). Phylogeny, adaptations, biogeography and classification. *Entomonograph*, vol. 3, no. 11, pp. 1-455.

ANDERSEN, N.M., 1995. Cladistic inference and evolutionary scenarios: locomotory structure, function, and performance in water striders. *Cladistics*, vol. 11, no. 3, pp. 279-295. http://dx.doi.org/10.1016/0748-3007(95)90016-0. PMid:32313362.

AZAM, I., AFSHEEN, S., SARWAR, M.K., ZIA, A. and BHATTI, A.R., 2017. Effect of heavy metals on antioxidant enzymes in *Oxya hyla hyla* (Orthoptera: acrididae). *Pakistan Entomologist*, vol. 39, no. 2, pp. 37-44.

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*, vol. 2015, pp. 942751. http://dx.doi.org/10.1155/2015/942751. PMid:26167507.

BARATA, C., LEKUMBERRI, I., VILA-ESCALÉ, M., PRAT, N. and PORTE, C., 2005. Trace metal concentration, antioxidant enzyme activities and susceptibility to oxidative stress in the tricoptera larvae *Hydropsyche exocellata* from the Llobregat river basin (NE Spain). *Aquatic Toxicology (Amsterdam, Netherlands)*, vol. 74, no. 1, pp. 3-19. http://dx.doi.org/10.1016/j.aquatox.2005.04.002. PMid:15916818.

CELINO, F.T., YAMAGUCHI, S., MIURA, C., OHTA, T., TOZAWA, Y., IWAI, T. and MIURA, T., 2011. Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. *PLoS One*, vol. 6, no. 2, pp. e16938. http://dx.doi.org/10.1371/journal.pone.0016938. PMid:21364994.

CHENG, L., 1974. Notes on the ecology of the oceanic insect Halobates. *Marine Fisheries Review*, vol. 36, pp. 1-7.

CROSS, C.E., HALLIWELL, B., BORISH, E.T., PRYOR, W.A., AMES, B.N., SAUL, R.L., MCCORD, J.M. and HARMAN, D., 1987. Oxygen radicals and human disease. *Annals of Internal Medicine*, vol. 107, no. 4, pp. 526-545. http://dx.doi.org/10.7326/0003-4819-107-4-526. PMid:3307585.

CUNY, D., VAN HALUWYN, C., SHIRALI, P., ZERIMECH, F., JÉRÔME, L. and HAGUENOER, J.M., 2004. Cellular impact of metal trace elements in terricolous lichen *Diploschistes muscorum* (Scop.) R. Sant. - Identification of oxidative stress biomarkers. *Water,* *Air, and Soil Pollution*, vol. 152, no. 1-4, pp. 55-69. http://dx.doi.org/10.1023/B:WATE.0000015332.94219.ff.

DAS, K., SAMANTA, L. and CHAINY, G.B.N., 2000. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. *Indian Journal of Biochemistry & Biophysics*, vol. 37, no. 3, pp. 201-204.

DUTTA, P., DEY, T., MANNA, P. and KALITA, J., 2016. Antioxidant potential of *Vespa affinis* L., a traditional edible insect species of North East India. *PLoS One*, vol. 11, no. 5, pp. e0156107. http://dx.doi.org/10.1371/journal.pone.0156107. PMid:27195807.

FELTON, G.W. and SUMMERS, C.B., 1995. Antioxidant systems in insects. *Archives of Insect Biochemistry and Physiology*, vol. 29, no. 2, pp. 187-197. http://dx.doi.org/10.1002/arch.940290208. PMid:7606043.

FINKEL, T., 2011. Signal transduction by reactive oxygen species. *The Journal of Cell Biology*, vol. 194, no. 1, pp. 7-15. http://dx.doi.org/10.1083/jcb.201102095. PMid:21746850.

GOVIND, P. and MADHURI, S., 2014. Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, vol. 2, no. 2, pp. 17-23.

ISLAM, S.J., MANNA, P., UNNI, B. and KAILTA, J., 2019. Higher concentrations of heavy metals impair antioxidant defense mechanism and growth response of muga silkworm, *Antheraea assamensis* (Lepidoptera: saturniidae). *Journal of Entomology and Zoology Studies*, vol. 7, no. 2, pp. 715-724.

JARDINE, T.D., AL, T.A., MACQUARRIE, K.T.B., RITCHIE, C.D., ARP, P.A., MAPRANI, A. and CUNJAK, R.A., 2005. Water striders (family Gerridae): mercury sentinels in small freshwater ecosystems. *Environmental Pollution*, vol. 134, no. 1, pp. 165-171. http://dx.doi.org/10.1016/j.envpol.2004.07.006. PMid:15572234.

KAKKAR, P., DAS, B. and VISWANATHAN, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics*, vol. 21, no. 2, pp. 130-132. PMid:6490072.

LEE, K.S., KIM, S.R., PARK, N.S., KIM, I., KANG, P.D., SOHN, B.H., CHOI, K.H., KANG, S.W., JE, Y.H., LEE, S.M., SOHN, H.D. and JIN, B.R, 2005. Characterization of a silkworm thioredoxin peroxidase that is induced by external temperature stimulus and viral infection. *Insect Biochemistry and Molecular Biology*, vol. 35, no. 1, pp. 73-84. http://dx.doi.org/10.1016/j.ibmb.2004.09.008. PMid:15607657.

LUCK, H., 1974. Catalase. In: H.U. BERGMEYER and M. GRABI, eds. *Methods of enzymatic analysis*. New York: Academic Press, pp. 885-890.

MASOOD, N., FAROOQI, A. and ZAFAR, M.I., 2019. Health risk assessment of arsenic and other potentially toxic elements in drinking water from an industrial zone of Gujrat, Pakistan: a case study. *Environmental Monitoring and Assessment*, vol. 191, no. 2, pp. 95. http://dx.doi.org/10.1007/s10661-019-7223-8. PMid:30673908.

MIGULA, P., ŁASZCZYCA, P., AUGUSTYNIAK, M., WILCZEK, G., ROZPĘDEK, K., KAFEL, A. and WOŁOSZYN, M., 2004. Antioxidative defence enzymes in beetles from a metal pollution gradient. *Biologia*, vol. 59, no. 5, pp. 645-654.

MISSIRLIS, F., PHILLIPS, J.P. and JÄCKLE, H., 2001. Cooperative action of antioxidant defense systems in Drosophila. *Current Biology*, vol. 11, no. 16, pp. 1272-1277. http://dx.doi.org/10.1016/S0960-9822(01)00393-1. PMid:11525742.

NASIR, A., ARSLAN, C., KHAN, M.A., NAZIR, N., AWAN, U.K., ALI, M.A. and WAQAS, U., 2012. Industrial waste water management in district gujranwala of pakistan-current status and future suggestions. *Pakistan Journal of Agricultural Sciences*, vol. 49, no. 1, pp. 79-85.

NATIONAL ENVIRONMENTAL QUALITY STANDARDS – NEQS, 2000. National Environmental Quality Standards for Municipal and Liquid Industrial Effluents of Pakistan. *The Gazette of Pakistan, Government of Pakistan*, vol. 1, S.R.O. 549.

NUMMELIN, M., LODENIUS, M. and TULISALO, E., 1998. Water striders (Heteroptera: Gerridae) as bioindicators of heavy metal pollution. *Entomologica Fennica*, vol. 8, pp. 185-191. http://dx.doi.org/10.33338/ef.83942.

PRITSOS, C.A., AHMAD, S., BOWEN, S.M., BLOMQUIST, G.J. and PARDINI, R.S., 1988. Antioxidant enzyme activities in the southern armyworm, *Spodoptera eridania*. *Comparative Biochemistry and Physiology. Part C, Comparative*, vol. 90, no. 2, pp. 423-427. http://dx.doi.org/10.1016/0742-8413(88)90021-7.

PÜTTER, J., 1974. Peroxidases. *Methods of Enzymatic Analysis*, vol. 2, pp. 685-690. http://dx.doi.org/10.1016/B978-0-12-091302-2.50033-5.

QURESHI, I.Z., KASHIF, Z., HASHMI, M.Z., SU, X., MALIK, R.N., ULLAH, K., HU, J. and DAWOOD, M., 2015. Assessment of heavy metals and metalloids in tissues of two frog species: *Rana tigrina* and *Euphlyctis cyanophlyctis* from industrial city Sialkot, Pakistan. *Environmental Science and Pollution Research International*, vol. 22, no. 18, pp. 14157-14168. http://dx.doi.org/10.1007/s11356-015-4454-2. PMid:25966879.

R CORE TEAM, 2017 [viewed 10 June 2020]. *R: a Language and Environment for Statistical Computing* [online]. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.r-project.org/

RAFIQUE, U., ASHRAF, A., KHAN, A., NASREEN, S., RASHID, R. and MAHMOOD, Q., 2010. Toxic chromium from tanneries pollute water resources and soils of Sialkot (Pakistan). *Journal of the Chemical Society of Pakistan*, vol. 32, no. 5, pp. 644-649.

REHMAN, W., ZEB, A., NOOR, N. and NAWAZ, M., 2008. Heavy metal pollution assessment in various industries of Pakistan. *Environmental Geology*, vol. 55, no. 2, pp. 353-358. http://dx.doi.org/10.1007/s00254-007-0980-7.

SCHIEBER, M. and CHANDEL, N.S., 2014. ROS function in redox signaling and oxidative stress. *Current Biology*, vol. 24, no. 10, pp. 1-25. http://dx.doi.org/10.1016/j.cub.2014.03.034. PMid:24845678.

SHARMA, P., JHA, A.B., DUBEY, R.S. and PESSARAKLI, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Le Journal de Botanique*, vol. 2012, pp. 1-26. http://dx.doi.org/10.1155/2012/217037.

SPENCE, J.R. and ANDERSON, N.M., 1994. Biology of water striders: interactions between systematics and ecology. *Annual Review of Entomology*, vol. 39, no. 1, pp. 101-128. http://dx.doi.org/10.1146/annurev.en.39.010194.000533.

ULLAH, R., MALIK, R.N. and ABDUL, Q., 2009. Assessment of groundwater contamination in an industrial city, Sialkot, Pakistan. *African Journal of Environmental Science and Technology*, vol. 3, no. 12, pp. 429-446.

WASEEM, A., ARSHAD, J., IQBAL, F., SAJJAD, A., MEHMOOD, Z. and MURTAZA, G., 2014. Pollution status of Pakistan: a retrospective review on heavy metal contamination of water, soil, and vegetables. *BioMed Research International*, vol. 2014, pp. 813206. http://dx.doi.org/10.1155/2014/813206. PMid:25276818.

WICKHAM, H., 2009. *ggplot2: elegant graphics for data analysis using the grammar of graphics*. New York: Springer-Verlag. https://doi.org/10.1080/15366367.2019.1565254.

WORLD HEALTH ORGANIZATION – WHO, 2011. *Guidelines for drinking-water quality*. 4th ed. Geneva: WHO, pp. 1-541.

WU, G. and YI, Y., 2015. Effects of dietary heavy metals on the immune and antioxidant systems of *Galleria mellonella* larvae. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, vol. 167, pp. 131-139. http://dx.doi.org/10.1016/j.cbpc.2014.10.004. PMid:25463648.

YANG, L.H., HUANG, H. and WANG, J.J., 2010. Antioxidant responses of citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), exposed to thermal stress. *Journal of Insect Physiology*, vol. 56, no. 12, pp. 1871-1876. http://dx.doi.org/10.1016/j.jinsphys.2010.08.006. PMid:20709071.

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 International*, vol. 26, no. 4, pp. 3823-3833. http://dx.doi.org/10.1007/s11356-018-3756-6. PMid:30539392.