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Heavy metal concentrations and its impact on soil microbial and enzyme activities in agricultural lands around ship yards in Chattogram, Bangladesh

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Abstract

The present endeavor was to evaluate the spatial distribution and ecological risk of heavy metals, released from ship scrap processing activities in agricultural soils of Sitakunda, Bangladesh. Soil samples were collected from 19 sites located in the vegetable garden, vegetable field and paddy field soils. The studied soils have the texture of sandy clay loam, extremely acidic to moderately acidic pH (4.23–5.88), soil organic matter was in the range from 0.79 to 1.43%. The mean concentrations of all the heavy metals were higher than the standard limit value. Heavy metal concentrations ranged from 1.77 to 8.10 mg·kg^{-1} Cd, 102.75 to 262.00 mg·kg^{-1} Cr, 90.52 to 662.33 mg·kg^{-1} Cu, 26.66 to 227.47 mg·kg^{-1} Ni, 148.33 to 1483.33 mg·kg^{-1} Pb and 270.37 to 1416.13 mg·kg^{-1} Zn. The toxicity level of heavy metals in agricultural soils was, in order of decreasing concentration: Cd > Pb > Cu > Zn > Ni > Cr. The principal component analysis evidenced that the heavy metal contaminants in agricultural soils may originate from the ship scrap dismantling and processing operations. All the heavy metals had shown a very high significant negative correlation with the number of cultivable bacteria and fungi, soil microbial biomass carbon, and microbial activity as well as the dehydrogenases, urease, acid phosphatase and arylsulfatase enzyme activities. Dehydrogenases activity was a very responsive enzymatic assay ($p < 0.001$) to ascertain the effect of contamination on the physiologically active soil microorganisms. The positively correlated quadratic relationship between metabolic quotient and heavy metal concentration designate adapted and metabolically less efficient microbial population developed due to long-term heavy metal pollution in these agricultural soils.

1. Introduction

Soil microbial and enzyme activities are the driving force controlling all biochemical activities in soil. These biological activities are strong indicators of soil productivity as they expeditiously react to environmental changes induced by pollution and contamination. An alteration of these activities may result in reduced soil quality (Wolińska et al., 2015). So, their estimation may provide useful information and be helpful to determine the effects of soil-specific environmental stress or management practices (Kuźniar et al., 2018). Some soil microbiological parameters: soil microbial biomass carbon (MBC), basal respiration (MA), bacterial families and genera (Wolińska et al., 2018) and enzyme activity (Akmal and Jianming, 2009) have been suggested as attainable indicators of soil quality and employed in the monitoring programs.

Soil pollution with heavy metals in different quantities and forms causes changes in the population and activity of microorganisms and enzymes, which is a true reflection of the actual microbiological condition of the soil (Kuźniar et al., 2018). Heavy metals can create abiotic stresses by inducing disorders in the metabolism of soil microorganisms when present in high concentrations. The results reported by Jiang et al., (2010) indicate that cadmium (Cd), copper (Cu) and zinc (Zn) can disrupt the microbiological equilibrium of soil. Many researchers (Kuźniar et al., 2018; Wyszkowska et al., 2008) demonstrated that Cd, Cu and Zn, when present in excessive quantities in soil, cause disorders in the microbiological balance of the soil. There is a very close relationship between soil enzymes and soil microbes as enzymes secreted by microorganisms regulate the energy and nutrient cycle in the soil ecosystems. Soil enzymes are involved in synthesizing proteins, carbohydrates and nucleic acids and reg-

ulate the decomposition of soil organic matter, releasing plant nutrients (Wolińska et al., 2015). Therefore, enzymatic activity can act as an indicator of soil health relating biological processes with physicochemical properties and stressed conditions which further can stipulate soil degradation. A high content of toxic heavy metals can inhibit the growth and reproduction of microorganisms by reducing the synthesis of the microbial enzyme or by modifying the enzyme-substrate complexes, enzyme protein and blocking active sites. When a metal enters the soil, it can alter the soil pH and usually results in acidification. Increasing heavy metal levels reduced soil enzyme activities were reported by Kuźniar et al., (2018).

Ship dismantling is initiated in Bangladesh in 1969 that experienced a boom in the 1980s and extends along the Sitakunda coast of Chattogram, Bangladesh. Ship dismantling activities and its scrap processing emerge many heavy metals that are found in many parts of ships such as in batteries, coatings, paints and electrical systems (Chowdhury and Rasid, 2016). Most of the ship wastes go to the informal sector as scrap and dumped beside workshops situated in the village vicinity of the shipyards for further processing. Monitoring is very strict in shipyards but the Government authority is not concerned about these workplaces. Ship scraps are scavenged for recycling by using primitive processes like unprotected acid leaching, manual dismantling and burning to recover worthwhile metals. Ship scrap processing sites are usually located in fields adjacent to land used for agricultural purposes. Heavy metals released could penetrate the soils where vegetables and rice are grown by contaminated irrigation water and through direct deposition by air, rainwater. Sitakunda is famous for agricultural products especially winter vegetables. Heavy metal contamination of these soils is of great concern as heavy metal contamination has a prolonged effect on soil ecology. Furthermore, an agricultural ecosystem has a close relationship with human health. Such distribution of heavy metals to the ambient area as well as on-site pollution may pose a direct agricultural and environmental hazard. The impact of hazardous substances, including heavy metals on beach soil and marine environment had been studied extensively (Alam et al., 2019; Rahman et al., 2019a, 2019b; Aktaruzzaman et al., 2014; Hasan et al., 2013). Similarly, higher concentrations of heavy metals such as cadmium, copper, lead, nickel and zinc were recorded in the adjacent areas of shipyards in Chattogram, Bangladesh (Chowdhury and Rasid, 2016; Alamgir et al., 2015), but there is no work on soil microbial and enzyme activity under this polluted area (Table 1). The present endeavor was to (a) compare the concentrations of metals determined, with background concentrations and standard limit values to show the extent of pollution (b) to assess the ecological risk by the heavy metals and (c) to determine the relationship of soil microbiological properties and four soil enzymatic activities (dehydrogenases, urease, acid phosphatase, arylsulfatase) from adjacent agricultural soils of ship dismantling area especially along the Dhaka-Chattogram highway to the heavy metal contamination, if there any. There are thousands of small unauthorized workshops, where opera-

Table 1
Comparison with reports of metal (Cd = Cadmium, Cr = Chromium, Cu = Copper, Ni = Nickel, Pb = Lead, Zn = Zinc) concentrations in Chattogram, Bangladesh.

Land use type	Cd	Cr	Cu	Ni	Pb	Zn	Reference
				mg·kg^{-1}			
Shipyard	5.50–9.30	250.80–160.00	180.20–155.90	90.80–67.00	103.10–134.60	713.30–883.10	Alam et al., 2019
	–	7.95–19.22	15.4–21.95	–	65.50–116.90	560.00	Rahman et al., 2019a
	0.26	94.90	86.90	53.00	339.70	560.00	Rahman et al., 2019b
	BDL	39.78–223.18	25.25–83.36	–	16.39–85.83	37.05–103.88	Aktaruzzaman et al., 2014
	0.01–1.16	311–1232	6.00–1635.00	8.00–45.00	16.00–22.00	58.00–978.00	Hasan et al., 2013
	0.55–3.95	0.60–65.20	BDL–295.65	16.30–162.20	BDL–137.05	33.25–305.10	Ahmed et al., 2013
	0.09–0.18	1.06–2.40	2.32–3.96	1.26–2.16	3.42–6.03	1.89–2.70	Hossain and Islam 2006
Agricultural	0.14–1.39	37.20–113.70	32.40–555.80	33.50–82.10	19.50–287.00	181.60–3648.40	Hasan et al., 2020
	0.21–6.20	10.70–190	28.81–919.02	9.36–120.00	148.70–1694.33	41.73–331.56	Chowdhury and Rasid 2016
City area	–	17.70–99.08	20.34–33.06	34.10–41.27	23.66–25.05	59.69–74.32	Wang et al., 2016
	0.52–4.84	–	4.68–74.33	13.17–2551.96	3.63–13.40	23.20–402.95	Alamgir et al., 2015
	0.50–1.20	–	37.00–42.00	–	70.00–82.00	248.00–317.00	Alam et al., 2012
Standard value for agricultural soil							
China	0.30	Dry soil 200.00 Paddy soil 150.00	100.00	50.00	300.00	250.00	Chen et al., 2018
Netherlands	0.80	100.00	36.00	35.00	85.00	140.00	VROM 2000
Canada	1.40	64.00	63.00	50.00	140.00	200.00	CCME 2003
Australia	3.00	50.00	60.00	60.00	300.00	200.00	DEC 2010

Explanation: BDL – Below detectable level.

tions of ship scrap processing are going on all year-round. Most uncontrolled ship scrap processing sites are located in or close to agricultural land where rice and vegetables are grown regularly. It is hypothesized that these soils may lack different nutrient cycling enzymes for high heavy metal contamination and that may also affect the soil quality and nutrient cycle in soil.

2. Materials and methods

2.1. Description of the study area

A segment of the area covering the villages beside the shipyards to the stalls selling the goods and scraps from ships and scrap processing workshops along with Dhaka-Chattogram highway in Sitakunda, Chattogram (22°37'N and 91°39.7'E longitude) (Fig. 1) was selected for the study. According to the United States Department of Agriculture (USDA) soil classification system, the soils analyzed in the study were classified as the Lithic Ustochrepts (Huq and Shoaib, 2013). The topography of Sitakunda is hill with elevations ranging from 50 to 150 m above sea level. There is a tropical monsoon climate (Misbahuzzaman and Alam, 2006) with an average maximum temperature is 32.3°C during May, and the minimum, 13.9°C in January. The annual average rainfall is 2890 mm. The active uncontrolled processing of ship scraps had left open incineration sites scattered among agricultural fields, and ship wastes dumped beside the ponds. Amid the ship scrap recycling activities, agricultural operations, such as planting rice and vegetables were taking place in the area. Spots for soil sampling were selected based on visual activities of ship scarp processing. Soil samples were collected from agricultural fields adjacent to ship scrapping operational sites. These areas were continuously receiving discharge from the working area. All of the sampling locations in the present study can be classified into three groups: vegetable field, paddy field around scrap processing or dumping sites and vegetable garden near the homestead area.

2.2. Soil sampling and processing

The soil samples (topsoil, 0–15 cm) were collected from nineteen sites (Table 2), including one comparison sample reference site (C), which was not polluted by heavy metals. From the vegetable garden, vegetable field and paddy fields, eight (VG1-VG8), six (VF1-VF6) and four (P1-P4) sites were selected respectively. Three soil subsamples, five kilograms each were collected from the sites. Soil sampling was done with a stainless-steel spade. All of the samples were put in polythene bags and transported to the laboratory on the day of sampling. The composite soil samples were sieved through a 2 mm sieve, homogenized, a portion of this was air-dried for physical and chemical analysis another portion was ground to pass through a 1 mm sieve, adjusting to 45% of water holding capacity, stored in polythene bags at 4°C before soil microbial and enzymatic activities analysis.

Fig. 1. Location of the study area (website 1)

Table 2
Site legend, locations and soil reaction (pH), soil organic matter (SOM), total nitrogen (TN), available phosphorus (AvP) and clay content (Clay) values (mean ±SD) of different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram.

Legend	Site	pH	SOM	TN	AvP	Clay	Soil Texture
			%	%	mg·kg^{-1}	%	
Vegetable garden (VG)							
VG1	Acid-leaching site	4.23±0.03^{a}	0.91±0.02^{bc}	0.10±0.01^{a}	5.95±0.56^{a}	27.41±0.25^{a}	Sandy clay loam
VG2	Burning site	4.35±0.01^{b}	0.93±0.00^{bc}	0.13±0.02^{b}	5.95±0.08^{a}	27.98±0.04^{b}	Sandy clay loam
VG3	Dismantling site	4.55±0.01^{c}	0.93±0.00^{bc}	0.13±0.01^{b}	6.95±0.05^{b}	28.13±0.06^{bc}	Sandy clay loam
VG4	Burnt plastic dump site	5.83±0.02^{p}	1.41±0.09^{h}	0.17±0.00^{fg}	12.18±0.02^{jk}	31.03±0.67^{i}	Sandy clay loam
VG5	Metal workshop/processing site	4.64±0.01^{e}	0.80±0.01^{a}	0.14±0.00^{bc}	8.08±0.03^{c}	28.61±0.09^{d}	Sandy clay loam
VG6	Electric waste dumping site	4.96±0.01^{k}	1.29±0.00^{g}	0.15±0.00^{cde}	10.15±0.13^{f}	29.06±0.04^{efg}	Sandy clay loam
VG7	Scrap dumping site	4.72±0.01^{g}	1.05±0.01^{ef}	0.14±0.00^{bcd}	8.53±0.13^{d}	28.85±0.04^{de}	Sandy clay loam
VG8	Waste oil processing site	4.87±0.02^{i}	0.90±0.01^{bc}	0.14±0.00^{bcd}	9.03±0.06^{e}	29.00±0.00^{efg}	Sandy clay loam
Vegetable field (VF)							
VF1	Burning site	4.80±0.02^{h}	0.89±0.00^{b}	0.15±0.00^{def}	11.34±0.02^{h}	28.94±0.05^{ef}	Sandy clay loam
VF2	Dismantling site	4.91±0.01^{j}	0.93±0.01^{bc}	0.18±0.01^{g}	10.54±0.13^{g}	29.00±0.00^{efg}	Sandy clay loam
VF3	Acid-leaching site	4.67±0.01^{f}	0.95±0.18^{bcd}	0.15±0.00^{def}	11.77±0.01^{k}	28.78±0.03^{de}	Sandy clay loam
VF4	Metal workshop	4.97±0.02^{k}	0.98±0.00^{bcde}	0.15±0.00^{cde}	11.40±0.05^{i}	29.10±0.00^{efg}	Sandy clay loam
VF5	Scrap dumping site	5.02±0.00^{l}	1.00±0.01^{cde}	0.14±0.02^{bcd}	11.73±0.11^{i}	29.23±0.12^{fg}	Sandy clay loam
VF6	Electric waste dumping site	5.11±0.02^{m}	1.03±0.03^{def}	0.15±0.00^{def}	12.05±0.05^{j}	29.32±0.02^{g}	Sandy clay loam
Paddy field sites (P)							
P1	Scrap dumping site	5.72±0.01^{n}	1.27±0.03^{g}	0.16±0.00^{ef}	12.25±0.05^{jk}	31.43±0.06^{j}	Sandy clay loam
P2	Electric waste dumping site	5.76±0.02^{o}	1.23±0.01^{g}	0.17±0.01^{g}	12.36±0.05^{k}	31.57±0.06^{j}	Sandy clay loam
P3	Metal workshop site	4.59±0.02^{d}	1.11±0.01^{f}	0.15±0.00^{bcde}	11.25±0.00^{h}	28.32±0.09^{c}	Sandy clay loam
P4	Product storage site	5.88±0.02^{q}	1.43±0.01^{h}	0.24±0.01^{i}	13.06±0.03^{m}	30.01±0.11^{h}	Sandy clay loam
Reference site (C)							
C	10 km away from the recycling site	5.96±0.04^{r}	1.51±0.02^{i}	0.21±0.01^{h}	12.78±0.08^{l}	29.82±0.03^{h}	Sandy clay loam
Land use types							
	Vegetable garden	4.77±0.47^{x}	1.03±0.20^{x}	0.14±0.02^{x}	8.35±2.05^{x}	28.76±1.05^{x}	Sandy clay loam
	Vegetable field	4.91±0.15^{x}	0.96±0.08^{x}	0.15±0.01^{x}	11.47±0.50^{y}	29.06±0.19^{xy}	Sandy clay loam
	Paddy field	5.49±0.54^{y}	1.26±0.12^{y}	0.18±0.04^{y}	12.23±0.68^{y}	30.33±1.37^{z}	Sandy clay loam
	Reference	5.96±0.04^{z}	1.51±0.02^{z}	0.21±0.01^{z}	12.78±0.08^{y}	29.82±0.03^{yz}	Sandy clay loam

Values in the same column followed by the same letter(s) are not significantly different at $p < 0.05$ according to ANOVA.

2.3. Analysis

General soil characteristics were determined following the standard procedures. The pH of the soil samples was measured by pH meter at dry soil and distilled water ratio of 1 : 5 as described in Jackson (1973). Particle size distributions of the soils were determined by the hydrometer method (Day, 1965). Textural classes were determined using “soil automatic texture calculator” by Natural Resources Conservation Service Soils of the United States Department of Agriculture (website 2). The organic carbon (OC) content of the soil samples was determined volumetrically by the wet oxidation method by Nelson and Sommers (1982). Organic matter content was estimated through the use of an approximate correction factor, the “Van Bemmelen factor” of 1.724 which is based on the assumption that organic matter contains 58 percent OC. Total nitrogen (TN) content in soil was determined by the Micro-Kjeldahl method following H_2SO_4 acid digestion and alkali distillation and available phosphorus (AvP) by the colorimetric method after digestion with hydrofluoric and perchloric acid (Jackson, 1973). The total concentrations of metals were determined by Atomic Absorption Spectrophotometer (Aligent 240) after strong acid digestion (1:1 mixture of concentrated nitric and perchloric acids) of 200 mg of soil samples. The digested samples were filtered and collected in 5 ml of 2.0 M HCL as in Ure (1990).

2.3.1. Measurement of soil microbiological properties

Numbers of total bacteria and fungi in soils were counted using the dilution plate method as described in Johnson and Curl (1972). Nutrient agar (NA) medium was used with bacteria and potato dextrose agar (PDA) medium with fungi. Three plates were used for each dilution. The plates were incubated at 28°C for 7–10 days and counting made for forming colonies. MBC was measured by the method described by Anderson and Ingram (1993). The microbial cells in soil were killed by fumigation with ethanol-free chloroform. Immediately after pre-incubation, duplicate portions of soil, 5 g for each were taken in falcon tubes. One set of samples was fumigated with ethanol-free chloroform for 24 h at 25°C in a sealed desiccator. Non fumigated set of samples in falcon tubes were capped and stored at 8°C. After fumigant removal, both fumigated and non-fumigated soils were extracted with freshly prepared 0.5 M potassium sulfate at 1:4 ratios and filtered. Dissolved OC in the extracts was determined after dichromate digestion by titrating with 0.03 M acidified ferrous ammonium sulfate. The amount of soil MBC was calculated from the difference between the extracted carbon from chloroform fumigated and non-fumigated samples. MA was determined by trapping the CO_2 in NaOH which were evolved from the soil during incubation in a closed system (Alef and Nannipieri, 1995). The trapped CO_2 was determined by measuring electrical conductivity (Rodella and Saboya, 1999). For this purpose, 50 g (oven-dry basis) moist pre-incubated (60% of water holding capacity for 10 days) soil was placed in 1-liter capacity incubation Jars. Ten ml of 1.0 M NaOH solution in 50 ml falcon tubes were placed in each jar as the CO_2 trap. A falcon tube with water was added into the jar to maintain the soil moisture. Jars were made airtight immediately. Two jars with 1.0 M NaOH but without soil were used as controls. All jars were incubated at 25°C. CO_2 absorbed in traps was analyzed at 1, 7, 14, 30 days of NaOH placement. Each time fresh NaOH solution (10 ml) was replaced to trap CO_2 for the next days. In this method CO_2 evolved from each sample was calculated as the difference between the initial and the CO_2 concentration after each measurement period. The substrate-induced respiration (SIR) of the soils was assessed according to the rate of the maximal initial respiration of the microorganism after the enrichment of the soils with 0.5% glucose (West and Sparling, 1986). Over the first 2 h, the increase in CO_2-C is proportional to the size of the initial MBC concentration. Respiration was determined by trapping the CO_2 in NaOH as in MA.

2.3.2. Measurement of soil enzyme activities

Soil dehydrogenase activity was determined by the procedure of Casida (1977). Soil samples were suspended in a triphenyl tetrazolium chloride solution and incubated for 6 h at 37°C. The triphenyl formazan (TPF) produced was extracted with methanol and measured photometrically at 485 nm. Urease activity was assayed according to the method of Tabatabai and Bremner (1972). After the addition of a buffered urea solution, soil samples were incubated for 2 h at 37°C. The filtrated solution was distilled with MgO. The produced NH_4^+-N was collected into a boric acid indicator solution and titrated with diluted H_2SO_4 to determine the NH_4^+-N. Acid phosphatases activity was measured using the method of Eivazi and Tabatabai (1977). After the addition of a buffered p-nitrophenyl phosphate solution (pH 6.5), soil samples were incubated for 1 h at 37°C. The p-nitrophenol released by phosphomonoesterase activity was extracted and colored with NaOH and was measured photometrically at 400 nm. Arylsulfatase activity was measured by the potassium p-nitrophenyl sulfate method (Tabatabai and Bremner, 1970). After the addition of a buffered potassium p-nitrophenyl sulfate (pH 5.8), soil samples were incubated for 1 h at 37°C. The P-nitrophenol released by phosphomonoesterase activity was extracted and colored with NaOH and was measured photometrically at 400 nm.

2.3.3. Ecological risk assessment for soil pollution

Pollution levels of Cd, Cu, Cr, Ni, Pb and Zn in the soil samples were evaluated using heavy metal indices, such as contamination factor (C_f^i), degree of contaminations (C_d), pollution load index (*PLI*), total load of extractable metals (TLM) and geo-accumulation index (I_{geo}), which are widely used to estimate the contamination levels of heavy metals in agricultural soils (Adimalla and Li, 2019).

2.3.3.1. *Contamination factor (C_f^i)*

The contamination factor may be defined as the ratio of the metal concentration in the soil to that of background value. According to the intensities of contamination, the levels of contamination may be divided into six categories (Table 3) (Islam et al., 2015). Thus, the C_f^i values show the enrichment of heavy metals in soils of a certain place.

$$C_f^i = \frac{C_{\text{Heavy metal}}}{C_{\text{Background}}} \quad (1)$$

where, C_f^i = Contamination factor, $C_{\text{Heavy metal}}$ = the content of the heavy metal in samples, $C_{\text{Background}}$ = the background value of the heavy metal.

2.3.3.2. *Potential Ecological Risk (PER) index*

The degrees of heavy metal contamination in agricultural soils can be evaluated with *PER* index. The sensitivity of the biological community can be expressed by it to the heavy metal stress and indicates the potential ecological risk caused by the overall heavy metal contamination. The equations which were used to calculate *PER* are as follows (Guo et al., 2010):

$$PER = \sum_{i=1}^{m} E_r^i \qquad C_f^i = \frac{C^i}{C_n^i} \qquad C_d = \sum_{i=1}^{n} C_f^i \qquad E_r^i = T_r^i \times C_f^i \quad (2)$$

where, *PER* = comprehensive potential ecological risk index, C_f^i = single heavy metal contamination factor, C^i = content of the heavy metal in samples, C_n^i = background value of the heavy metal, C_d = degree of contaminations, E_r^i = potential ecological risk index, T_r^i = biological toxic factor, the biological toxic factors for cadmium = 30, chromium = 2, copper = 5, nickel = 6, lead = 5, and zinc = 1 (Guo et al., 2010, Islam and Hoque, 2014).

Table 3
Indices and grades of potential ecological risk of heavy metal pollution (Islam et al., 2015)

Potential Ecological Risk index (*PER*)				Degree of Contamination (C_d)				Geo-accumulation index (I_{geo})	
E^i_r	Grade	*PER*	Grade	C^i_f	Degree	C_d	Degree	I_{geo}	Degree
$E^i_r < 40$	Low	RI < 65	Low	$C^i_f < 1$	Low	$C_d < 5$	Low	$I_{geo} < 0$	Practically uncontaminated
$40 \le E^i_r < 80$	Moderate	65 ≤ RI < 130	Moderate	$1 \le C^i_f < 3$	Moderate	$5 \le C_d < 10$	Moderate	$0 < I_{geo} < 1$	Uncontaminated to moderately contaminated
$80 \le E^i_r < 160$	Considerable	130 ≤ RI < 260	Considerable	$3 \le C^i_f < 6$	Considerable	$10 \le C_d < 20$	Considerable	$1 < I_{geo} < 2$	Moderately contaminated
$160 \le E^i_r < 320$	High	RI ≥ 260	Very high	$C^i_f \ge 6$	High	$C_d \ge 20$	High	$2 < I_{geo} < 3$	Moderately to heavily contaminated
$E^i_r \ge 320$	Very high							$3 < I_{geo} < 4$	Heavily contaminated
								$4 < I_{geo} < 5$	Heavily to extremely contaminated
								$I_{geo} > 5$	Extremely contaminated

2.3.3.3. *Pollution Load Index (PLI)*

The pollution load index (*PLI*) acts as an integrated approach that expresses soil quality with the response to the heavy metals. The *PLI* is a calculation as the nth root of the multiplications of the contamination factor (C^i_f) of heavy metals (Suresh et al., 2015). The *PLI* value of zero indicates perfection, a value of one indicates the presence of only the baseline level of pollutants and values above one would indicate progressive deterioration of the soil quality (Proshad et al., 2019).

$$PLI = \left(C^i_{f1} \times C^i_{f2} \times C^i_{f3} \times \ldots \times C^i_{fn}\right)^{1/n} \quad (3)$$

where, *PLI* = pollution load index, C^i_f = single heavy metal contamination factor.

2.3.3.4. *Total Load of Extractable Metals (TLM)*

The total load of extractable metals (TLM) in soil was calculated for each site as follows (Simona et al., 2004):

$$TLM_j = \frac{Cd_m}{Cd_{\max}} + \frac{Cr_m}{Cr_{\max}} + \frac{Cu_m}{Cu_{\max}} + \frac{Pb_m}{Pb_{\max}} + \frac{Ni_m}{Ni_{\max}} + \frac{Zn_m}{Zn_{\max}} \quad (4)$$

where, TLM_j = total load of extractable metals measured in the jth site, X_m = measured value of the heavy metal X at each jth site, X_{max} = maximum value of the element X measured in the n_s sites, n_s = number of compared sites.

2.3.3.5. *Geo-accumulation Index (I_{geo})*

Geo-accumulation index (I_{geo}) is considered an effective tool to characterize the level of pollution from soil from the hazardous element (Proshad et al., 2018). I_{geo} was originally introduced and defined by Muller (1969). Geo-accumulation index (I_{geo}) can be determined by the following equation:

$$I_{geo} = C_n/(1.5 \times B_n) \quad (5)$$

where, I_{geo} = geo-accumulation index, C_n = measured concentration of metal n in the soil, B_n = geochemical background value of the element in the background sample. Factor 1.5 is introduced to minimize the possible variations in the background values which may be attributed to lithogenic effects (Yu et al., 2012). The interpretation of geo-accumulation index (I_{geo}) values is shown in Table 3.

2.3.4. Ecophysiological indices

Stress in the microbial population can be determined by the microbial quotient (*q*Mic) and metabolic quotient (qCO_2). Organic carbons in soil generally undergo microbial synthesis and are converted to humus. But, in the case of increased stress, more CO_2-carbon per unit microbial biomass per unit time is produced to counter stress.

2.3.4.1. *Metabolic Quotient (qCO_2)*

The metabolic quotient *(qCO_2)* was calculated from basal respiration at the end of the 30 days' incubation period according to the following equation (Anderson and Domsch, 1990).

$$qCO_2 = \frac{r}{MBC} \quad (6)$$

where, r = respiration rate, mg CO_2-C day^{-1} g^{-1} soil, MBC = soil microbial biomass carbon, mg C g^{-1} soil.

2.3.4.2. *Microbial Quotient (qMic)*

The microbial quotient (*q*Mic) representing the ratio of soil MBC to organic carbon expressed as percent (%) to explore the percent of organic carbon present as microbial biomass carbon (Anderson and Domsch, 1989):

$$q\text{MIC} = \frac{\text{MBC}}{\text{OC}} \quad (7)$$

where, *q*Mic = microbial quotient, MBC = soil microbial biomass carbon, μg, OC = total organic carbon, μg.

2.3.5. Ecological model for soil enzyme activity (ED_{50})

The inhibition of enzymatic activity by heavy metal was assessed by two kinetic models (Model 1: Equation 8 and Model 2: Equation 9) and a sigmoidal dose-response model (Model 3: Equation 10) using potential ecological risk index (*PER*) (Gao et al., 2010). The 50% ecological dose (ED_{50}) values are calculated for Models 1 and 2 by fitting Equation 11 and for Model 3 by fitting the Equation 12:

$$\text{Model 1}: \text{v} = \frac{c}{1 + bPER} \quad (8)$$

$$\text{Model 2}: \text{v} = \frac{c(1 + aPER)}{1 + bPER} \quad (9)$$

$$\text{Model 3}: \text{v} = \frac{x}{1 + e^{y(l-z)}} \quad (10)$$

$$\text{ED}_{50} = \frac{1}{b} \quad (11)$$

$$\text{ED}_{50} = e^{c} \quad (12)$$

where, ED_{50} = total ecological toxicity coefficients which lead to enzyme activity inhibited by 50%, *PER* = potential ecological risk index under multiple heavy metal pollution in Equation 2, v = response variable, a, b and c = fitting parameters with positive values and b > a, l = natural logarithm of *PER*, x = uninhibited value of v, y = slope factor, z = natural logarithm of ED_{50}.

2.3.6. Statistical analyses

All the measurements were made in triplicate soil samples and the results are expressed on an oven-dry weight basis. Correlations between the selected parameters, level of significance and standard deviation were determined using statistical packages in Office 2016 Program. The effects of different heavy metals were determined by one-way analysis of variance (ANOVA) with three replicates and the significance of the parameters was tested using the least significant difference multiple range test at $p \leq 0.05$ after one-way ANOVA. Pearson's correlation coefficient analyses were carried out with IBM SPSS (version 20.0, Chicago, USA) to study relationships between soil physicochemical properties, heavy metal contents, soil microbial activities and enzyme activities. Pared-samples T-test measured for soil samples firstly by considering soil samples all together (n = 56), secondly by considering mean values representing the sites with different activities (n = 19) and thirdly considering site-specific field types (n = 4). Regression between soil parameters and soil microbial activities and enzyme activities was fitted to linear and exponential functions. The Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) were performed by XLSTAT.

3. Results and discussion

3.1. Heavy metal contents of the soils

Heavy metal concentrations in the agricultural soil samples of different land-use sites showed significant variability ranged from 1.77 to 8.10 mg·kg^{-1} Cd, 102.75 to 262.00 mg·kg^{-1} Cr, 90.52 to 662.33 mg·kg^{-1} Cu, 26.66 to 227.47 mg·kg^{-1} Ni, 148.33 to 1483.33 mg·kg^{-1} Pb and 270.37 to 1416.13 mg·kg^{-1} Zn (Table 4). Heavy metal concentrations in the study region were also compared to the standard values for agricultural soil. As Bangladesh does not have any soil standards for heavy metals, standards developed in China (Chinese Environmental Quality Standards for Soil (Act No. 220/2004 Coll. of Laws) for agricultural soil were used to determine the extent of heavy metal contamination (Table 1). The values of heavy metals in the reference site were within the standard values. Compared with the standard values for agricultural soils, more than 50% of the mean values of the heavy metals especially Cd and Zn were above the standard values in the vegetable garden and vegetable field soils. The mean values of heavy metal contents in soils follow in decreasing order as Pb > Zn > Cd > Cu > Cr > Ni. The degree of heavy metal contamination among sampling sites generally followed the order: acid-leaching site> burning site> dismantling site> metal workshop> scrap dumping site> waste oil processing site> electric waste dumping site> burnt plastic dump site> product storage site. The heavy metal concentration of the soil samples collected from the contaminated sites showed consistency between the sites about the heavy metal concentrations. The sites that have higher concentrations have all the heavy metals in high concentrations.

To evaluate the data on heavy metal concentration, the descriptive statistics were calculated (Table 4). The skewness values for Cr was low and negative (–0.26), however, those for Cd, Cu, Ni, Pb and Zn were positively skewed with skewness values of 0.94, 1.04, 0.86, 1.11, and 0.71, respectively, indicating non-normality of the data set for these heavy metals. The calculated coefficient of variation (CV) varied from 34.31% to 75.01%, indicating moderate variation, expressing heterogeneous occurrence (Zhou et al., 2016). It also indicates that the sources of heavy metal were not natural, the sources were anthropogenic. As the workshops of the ship dismantling activities were not structured or planned around the village so there was no uniform distribution of the heavy metals in the area. Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the present study were also compared to other studies conducted in Bangladesh (Table 1). All the investigated heavy metal concentrations were higher than other studies with agricultural and urban soils of

Table 4

Descriptive statistics of heavy metals (Cd = Cadmium, Cr = Chromium, Cu = Cupper, Ni = Nickel, Pb = Lead, Zn = Zinc) concentration (mean ±SD) in soils of different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram

Descriptive statistics	Cd	Cr	Cu	Ni	Pb	Zn
	mg·kg^{-1}					
Vegetable garden site						
VG1	8.10 ±0.01^{a}	262.00 ±8.87^{a}	662.33 ±6.24^{a}	227.47 ±8.15^{a}	1483.33 ±76.38^{a}	1416.13 ±3.96^{a}
VG2	7.48 ±0.05^{b}	240.37 ±1.27^{b}	505.74 ±12.97^{b}	192.00 ±12.56^{b}	1109.00 ±8.54^{b}	966.07 ±23.79^{b}
VG3	5.34 ±0.03^{c}	225.89 ±1.83^{c}	457.05 ±28.48^{c}	162.89 ±4.34^{c}	1106.37 ±5.52^{b}	926.49 ±22.95^{c}
VG4	1.93 ±0.01^{p}	107.01 ±1.11^{m}	94.83 ±3.72^{o}	28.73 ±1.47^{n}	158.47 ±2.01^{l}	294.60 ±7.83^{n}
VG5	4.60 ±0.03^{e}	191.47 ±0.54^{d}	347.73 ±2.70^{e}	141.56 ±6.99^{e}	716.66 ±19.07^{d}	777.78 ±10.18^{e}
VG6	2.78 ±0.02^{k}	145.15 ±2.64^{i}	144.73 ±1.79^{k}	47.60 ±3.70^{k}	352.94 ±33.91^{h}	533.57 ±7.55^{j}
VG7	4.10 ±0.01^{g}	177.71 ±2.16^{e}	275.16 ±1.12^{g}	100.06 ±1.64^{g}	560.24 ±2.44^{e}	691.75 ±49.11^{f}
VG8	3.18 ±0.01^{i}	164.54 ±1.34^{g}	201.59 ±11.10^{i}	74.89 ±3.89^{i}	432.03 ±1.06^{g}	603.37 ±5.34^{hi}
Minimum	1.92	105.77	91.34	27.67	156.17	286.01
Maximum	8.11	268.90	668.59	236.65	1550.00	1420.16
Arithmetic mean	4.69^{x}	189.27^{x}	336.15^{x}	121.90^{x}	739.88^{x}	776.22^{x}
Median	4.34	185.24	310.55	117.96	629.17	745.80
SD	2.10	49.48	186.75	67.79	433.93	322.72
CV%	44.74	26.14	55.56	55.61	58.65	41.58
Vegetable field site						
VF1	3.46 ±0.06^{h}	169.92 ±0.80^{f}	241.29 ±22.51^{h}	87.66 ±3.09^{h}	513.99 ±19.93^{f}	620.13 ±3.54^{h}
VF2	2.84 ±0.03^{j}	154.96 ±1.60^{h}	165.24 ±4.80^{j}	58.26 ±1.79^{j}	353.89 ±7.02^{h}	584.52 ±21.44^{i}
VF3	4.25 ±0.05^{f}	182.23 ±4.57^{e}	313.60 ±3.74^{f}	128.59 ±6.26^{f}	551.61 ±63.52^{ef}	660.16 ±29.67^{g}
VF4	2.64 ±0.02^{l}	141.50 ±0.50^{i}	133.97 ±1.84^{kl}	40.76 ±0.85^{kl}	317.76 ±2.32^{hi}	517.44 ±6.82^{jk}
VF5	2.37 ±0.02^{m}	135.83 ±1.56^{j}	127.90 ±2.53^{lm}	37.80 ±0.45^{lm}	285.59 ±1.73^{ij}	501.98 ±1.82^{k}
VF6	2.25 ±0.02^{n}	123.79 ±1.29^{kl}	106.87 ±4.21^{no}	32.54 ±0.54^{mn}	230.40 ±2.64^{k}	414.63 ±15.60^{l}
Minimum	2.22	122.30	102.86	32.03	227.50	396.62
Maximum	4.30	187.47	317.46	134.45	624.05	679.45
Arithmetic mean	2.97^{y}	151.37^{xy}	181.47^{y}	64.27^{y}	375.54^{y}	549.81^{xy}
Median	2.74	147.85	149.25	48.74	334.76	541.94
SD	0.72	20.70	75.58	35.19	123.29	85.10
CV%	24.11	13.67	41.65	54.75	32.83	15.48
Paddy field site						
P1	2.28 ±0.02^{n}	128.00 ±1.00^{k}	113.01 ±2.06^{mn}	34.38 ±2.28^{lmn}	252.33 ±18.50^{jk}	490.73 ±4.95^{k}
P2	2.12 ±0.03^{o}	121.00 ±2.00^{l}	99.78 ±0.55^{no}	30.83 ±0.29^{mn}	222.72 ±4.93^{k}	352.64 ±3.53^{m}
P3	4.81 ±0.01^{d}	195.45 ±0.87^{d}	387.24 ±5.05^{d}	154.37 ±3.33^{d}	880.33 ±18.57^{c}	848.90 ±19.77^{d}
P4	1.77 ±0.02^{q}	102.75 ±1.71^{m}	90.52 ±0.71^{o}	26.66 ±0.67^{n}	148.33 ±5.40^{l}	270.37 ±12.27^{n}
Minimum	1.75	101.26	90.05	26.00	143.25	258.13
Maximum	4.82	196.36	392.77	157.11	900.00	871.55
Arithmetic mean	2.74^{y}	136.80^{y}	172.64^{y}	61.56^{y}	375.93^{y}	490.66^{y}
Median	2.21	124.65	105.70	31.61	228.40	422.03
SD	1.26	36.68	129.70	56.07	306.95	231.38
CV%	46.03	26.81	75.13	91.08	81.65	47.16
Reference site						
	0.28	23.45	23.75	25.67	8.35	22.67
C	0.27	17.69	23.17	27.67	10.00	23.87
	0.27	25.14	20.00	26.00	9.75	23.73
Minimum	0.27	17.69	20.00	25.67	8.35	22.67
Maximum	0.28	25.14	23.75	27.67	10.00	23.87
Arithmetic mean	0.27^{z}	22.09^{z}	22.31^{z}	26.44^{y}	9.37^{z}	23.42^{z}
Median	0.27	23.45	23.17	26.00	9.75	23.73
SD	0.01	3.91	2.02	1.07	0.89	0.66
CV%	2.20	17.68	9.05	4.05	9.49	2.81
All agricultural sites						
Range	7.84	251.21	648.59	210.98	1541.65	1397.49
Minimum	0.27	17.69	20.00	25.67	8.35	22.67
Maximum	8.11	268.90	668.59	236.65	1550.00	1420.16
Mean						
Statistic	3.50	157.46	236.36	85.97	509.76	604.98
Std. Error	0.25	7.16	22.13	8.27	50.64	39.73
Std. Deviation	1.91	54.03	167.10	62.47	382.35	299.92
Skewness						
Statistic	0.94	–0.26	1.04	0.86	1.11	0.71
Std. Error	0.32	0.32	0.32	0.32	0.32	0.32
Kurtosis						
Statistic	0.62	0.70	0.33	–0.49	0.50	1.31
Std. Error	0.62	0.62	0.62	0.62	0.62	0.62

Each mean is the average of the values obtained for three samples of each soil. Values in the same column followed by the same letter(s) are not significantly different at $p<0.05$ according to ANOVA. x,y,z for variation in land use types. Sampling sites legend description in Table 2.

Chattogram (Hasan et al., 2020) but consistent with the heavy metal content of sediments of shipyards (Alam et al., 2019; Rahman et al., 2019a; Chowdhury and Rasid, 2016).

The acid-leaching site and burning site of both vegetable garden and field soils showed a relatively high concentration of Cr and Ni compared to other sites. The elevated levels of Cr and Ni may be resulted from waste residues from acid leaching, burning and dismantling sites. Alarmingly, the concentrations of Cu in all the sampling sites were 2–13 times higher than the standard limit. The concentration of Cu in sample VG1 (662.33 $mg \cdot kg^{-1}$), collected from the vegetable garden nearby an acid leaching workshop, was the highest detected concentration in the agricultural field soils. The exception was Pb, for which the concentration was high and above the standard level in all the sites other than the burnt plastic zone in VG, e-waste zone in VF and paddy field and storage site near paddy fields. In general, there were highly significant (0.92 to 0.99%, $p < 0.05$) positive significant linear relation between various pairs of metals, reflecting their simultaneous release of an identical source or activity from the shipyard zone, transport and accumulation in soil (Ali et al., 2016). Differences in the recycling activities in each site may influence the distribution patterns of these metal pollutants.

A disproportionally high concentration of all the heavy metals in burning and acid-leaching sites indicates that point source pollutions existed in the sampling area. Irrigation can be a major pathway because the paddy and vegetable fields were irrigated with this untreated pond water and wastewater which was contaminated with different forms of Cr, Pb, Ni and Zn. Running off of acid rainwater from burning site (VG2, VF1), burnt plastic dump site (VG4), electric waste dumping site (VG6, VF6, P2), scrap dumping site (P1) carrying different levels of heavy metals can also be a source of heavy metal to the vegetable field and paddy field. Toxic heavy metals Pb, Ni, Cd, and Cr of sulfates, nitrates and chlorides are present in rainwater. E-waste contains Cd, Cu, Cr and Ni (Adesokan et al., 2016) because waste segregation is not common in Bangladesh. The workshop dust seems to carry the most serious toxic metals, followed by the open burning site soil and the dumpsite soil. Anoxic conditions in the paddy soil during the flooded period can esteem the formation of insoluble cadmium sulfide (Adesokan et al., 2016) and dissolution of Fe–Mn oxyhydroxides by releasing of adsorbed metals such as Pb and Zn (Rieuwerts et al., 1998). This could also explain why Cd and Zn accumulated in the paddy soil. The content of heavy metal may increase with operation time. Heavy metal concentration in some agricultural sites with similar activities (acid leaching, burning, dismantling and metal workshop) can be varied due to workshop operation time and structure. Brick structured workshops designed for scrap metal processing caused less heavy metal emission than unstructured open metal workshops. The agricultural soils beside the workshops along the Dhaka- Chattogram highway which run for decades were polluted more seriously.

3.1.1. Ecological risk assessment of heavy metals in the study area

The *PER* which shows the extent of contamination of the sampling sites indicated very high contamination of all the sampling sites. However, no potential ecological risk was found with reference sites, for which the sum of toxic units was lower than 40. In most soil samples, the E_r^i factors of Cd ranged above 160, and contamination levels were high risk to very high risk. The C_d and *PLI* followed a similar pattern of contamination result as for *PER* and suggest that agricultural soils in the area under investigation were very highly polluted with heavy metals (Fig. 2). Regarding the *PER*, VG1 and VG2 sites were moderate to considerably under risk with Ni and Zn. The ecological risk of Pb in the burning and acid-leaching sites was very high and Cu was at a considerable level. Among the land uses statistically significant differences ($p < 0.05$) were observed for the *PER* of heavy metal, which indicated that the combined ecological risk of heavy metals can vary with the impact of scrap processing activities along with different land uses (Table 5). The *PER* of the environment for the different types of land use can be ranked in the following order: vegetable garden> vegetable field> paddy field>reference site.

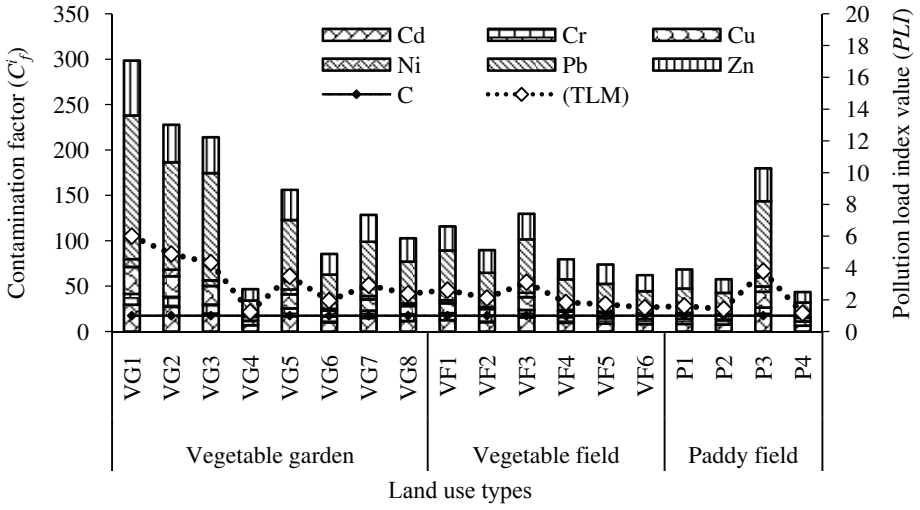

Fig. 2. Contamination factor (C_f^i), degree of Contamination (C_d) and pollution load index (*PLI*) value of heavy metals (Cd = Cadmium, Cr = Chromium, Cu = Cupper, Ni = Nickel, Pb = Lead, Zn = Zinc) in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram. Sampling sites legend description in Table 2

Table 5
Potential ecological risk factor (E^i_r), potential ecological risk index (*PER*) and pollution degree of heavy metals in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram

Sampling sites	Potential ecological risk factor (E^i_r)						Potential ecological risk	Pollution degree
	Cd	Cr	Cu	Ni	Pb	Zn		
VG1	**888.82**^{a}	23.72^{a}	148.47^{a}	51.61^{a}	**791.81**^{a}	60.46^{a}	**1964.89**^{a}	Very high
VG2	**820.29**^{b}	21.76^{b}	113.37^{b}	43.56^{b}	**591.99**^{b}	41.25^{b}	**1632.22**^{b}	Very high
VG3	**585.81**^{c}	20.45^{c}	102.45^{c}	36.96^{c}	**590.59**^{b}	39.56^{c}	**1375.81**^{c}	Very high
VG4	211.50^{p}	9.69^{m}	21.26^{o}	6.52^{n}	84.59^{l}	12.58^{n}	**346.13**^{n}	Very high
VG5	**504.27**^{e}	17.33^{d}	77.95^{e}	32.12^{e}	**382.56**^{d}	33.21^{e}	**1047.43**^{e}	Very high
VG6	305.45^{k}	13.14^{i}	32.44^{k}	10.80^{k}	188.40^{h}	22.78^{j}	**573.01**^{i}	Very high
VG7	**449.76**^{g}	16.09^{e}	61.68^{g}	22.70^{g}	299.06^{e}	29.53^{f}	**878.83**^{f}	Very high
VG8	**348.98**^{i}	14.89^{g}	45.19^{i}	16.99^{i}	230.62^{g}	25.76h^{i}	**682.44**^{h}	Very high
VF1	**379.21**^{h}	15.38^{f}	54.09^{h}	19.89^{h}	274.37^{f}	26.48^{h}	**769.41**^{g}	Very high
VF2	311.56^{j}	14.03^{h}	37.04^{j}	13.22 ^{j}	188.91^{h}	24.96^{i}	**589.71**^{i}	Very high
VF3	**466.51**^{f}	16.50^{e}	70.30^{f}	29.18^{f}	294.45^{ef}	28.19^{g}	**905.11**^{f}	Very high
VF4	289.25^{l}	12.81^{i}	30.03^{kl}	9.25^{kl}	169.62^{hi}	22.09^{jk}	**533.05**^{j}	Very high
VF5	259.88^{m}	12.30^{j}	28.67^{lm}	8.58^{lm}	152.45^{ij}	21.43^{k}	**483.30**^{k}	Very high
VF6	246.47^{n}	11.21k^{l}	23.96^{no}	7.38^{mn}	122.99^{k}	17.70^{l}	**429.70**^{lm}	Very high
P1	250.12^{n}	11.59^{k}	25.33^{mn}	7.80l^{mn}	134.70^{jk}	20.95^{k}	**450.49**^{l}	Very high
P2	232.04^{o}	10.95^{l}	22.37^{no}	6.99^{mn}	118.89^{k}	15.06^{m}	**406.30**^{m}	Very high
P3	**528.04**^{d}	17.69^{d}	86.80^{d}	35.03^{d}	**469.93**^{c}	36.24^{d}	**1173.73**^{d}	Very high
P4	193.84^{q}	9.30^{m}	20.29^{o}	6.05^{n}	79.18^{l}	11.54^{n}	**320.21**^{n}	Very high
C	30.00^{r}	2.00^{n}	5.00^{p}	6.00^{n}	5.00^{m}	1.00^{o}	49.00^{o}	Low

Vertically letters show statistically significant differences at ($p < 0.05$) among the land uses of each element. Bold indicates very high ecological risk. Sampling sites legend description in Table 2.

3.1.2. Geo-accumulation Index (I_{geo})

The I_{geo} is an effective numerical model, which has widely been used to evaluate the heavy metal contamination in agricultural soils (Adimalla and Li, 2019). The classification of I_{geo} given by Yu et al., (2012) is shown in Table 3 and its distribution is presented in Fig. 3. The I_{geo} values of all the heavy metals were found higher than 0 around the agricultural soils of the ship scrap processing sites, indicating the moderately to heavily contaminated soils by heavy metals. They indicate the contamination of soils caused by anthropogenic sources. The ranking of I_{geo} value is Pb > Zn > Cu > Cr > Cd > Ni.

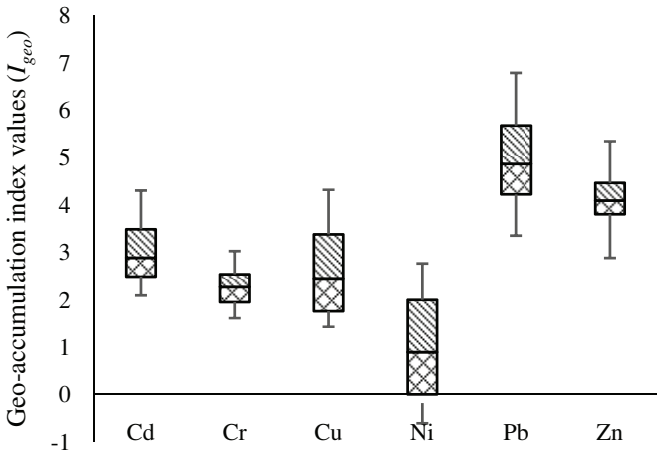

Fig. 3. Box and whisker plots display the distribution of the Geo-accumulation index (I_{geo}) value of heavy metals (Cd = Cadmium, Cr = Chromium, Cu = Cupper, Ni = Nickel, Pb = Lead, Zn = Zinc) in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram (error bar represents ±SD)

3.1.3. Source analysis of heavy metals under the study area

The PCA was performed to study the relationship between soil heavy metals, heavy metal indices and ship scrap processing sites. The result shows that the first and second principal components (PC1 and PC2) accounted for 57.07% and 17.57% of the total variance, respectively (Fig. 4).

The soil heavy metals and heavy metal indices were positively related to the acid leaching, dismantling, burning sites and negatively related reference site implying that the by product of acid leaching and dismantling of ship scrap was one of the major sources for heavy metal contamination. Acid leaching activities increased the heavy metal content in the soil. A high relative similarity was observed between the dismantling site and burning site soil and Cd, Cu, Ni and Zn and the distribution pattern of Cd and Cu were reasonably similar; this may imply that these heavy metals were mainly released from these two sites.

3.2. Effect of heavy metals on soil microbial number and activity

Significant variations have been observed in the microbial properties of soils of the studied area. The number of cultivable bacteria and fungi was found to be significantly low in agricultural soils than in reference soil. The number of cultivable bacteria and fungi in agricultural sites varied from 85×10^5 to 448×10^5 CFU·g^{-1} dry soil and 161×10^3 to 686×10^3 CFU·g^{-1} dry soil respectively. SIR, MA and MBC showed significant differences between agricultural sites and reference site. Microbial properties in the agricultural soil samples ranged from 28.49 to 68.45 mg CO_2·g^{-1} for SIR, 10.42 to 32.33 mg CO_2-C·g^{-1} day^{-1} for MA and 134.37 to 567.40 mg C·kg^{-1} for MBC. The mean of microbial properties in reference site soil was 72.25 mg CO_2·g^{-1} for SIR, 42.17 mg CO_2-C·g^{-1} day^{-1} for MA, 982.67 mg C·kg^{-1} for MBC respectively (Table 6). The microbial properties were also varied among the sampling sites of different land-use types and followed the descending order of paddy, vegetable field and vegetable garden soils. There were more than 80% fewer of cultivable bacteria and fungi and MBC in the acid-leaching site, burning site, dismantling site of VG and metal workshop site of paddy field due to ship scrap processing activities related to the reference site. The value of *q*Mic varied from 0.25–0.81 % in the agricultural soils. The negative effect of shipbreaking activities on soil microbiological properties in the contaminated soils has been revealed from the result as all the microbial parameters were significantly lower than in the reference site soils.

The soil microbial properties of the present study showed strong interrelations among themselves which is visible from Pearson's correlation coefficients (Fig. 5). Microbial population (bacteria and fungi) and SIR, microbial quotients were positively correlated with the MBC in soils and the correlation was significant at 0.05% level. High correlations between SIR and MBC (r = 0.82) indicate microbes highly respond with added glucose i.e. substrate, especially bacteria in the contaminated soils (Fig. 5). Correlations of bacteria with microbial activity (r = 0.88) and with MBC (r = 0.84, significant at 0.05% level) indicate more activeness and greater contribution of bacteria to MBC than fungi in contaminated soils.

Microbial activities found to be decreased in the highly contaminated agricultural soils and significant negative relations were found between soil heavy metal contents and microbial

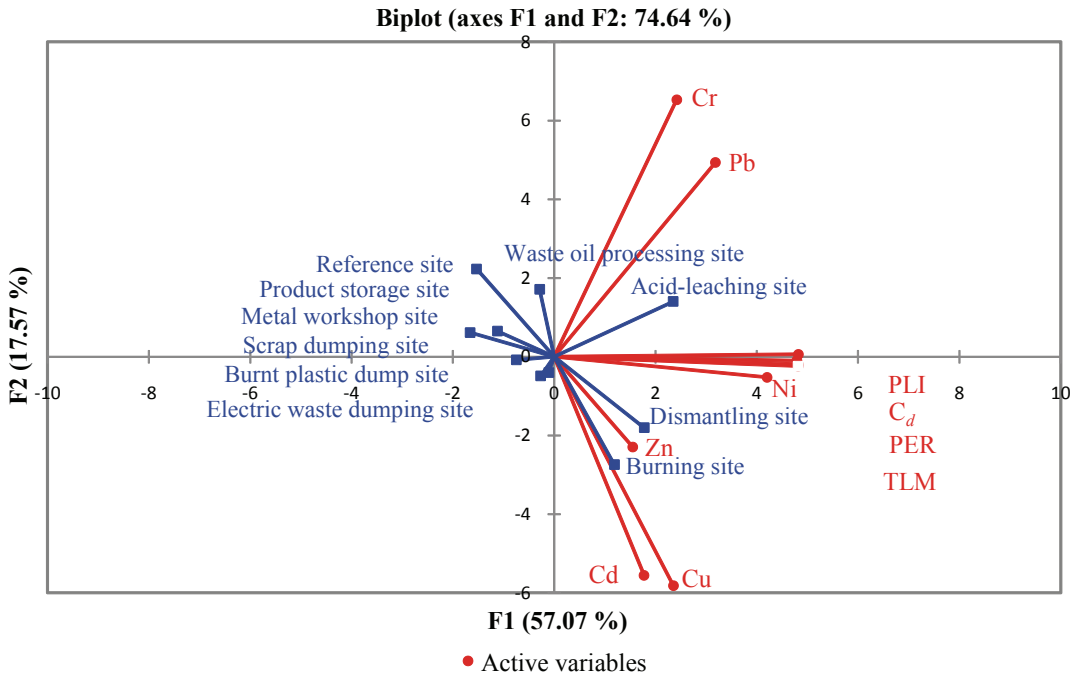

Fig. 4. Principal component analysis (PCA) plot showing the similarity of heavy metal (Cd = Cadmium, Cr = Chromium, Cu = Cupper, Ni = Nickel, Pb = Lead, Zn = Zinc) concentrations and heavy metal indices (*PLI* = Pollution load index, C_d = Integrated pollution degree, *PER* = Potential ecological risk, TLM = Total load of extractable metals) among sampling sites

Table 6
The number of cultivable bacteria (Bacteria), number of cultivable fungi (Fungi), substrate-induced respiration (SIR), microbial biomass carbon (MBC), mineralization quotient (*q*Mic) and metabolic quotient (qCO_2) (mean ±SD) in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram

Legend	Bacteria	Fungi	SIR	MA	MBC	*q*Mic	qCO_2
	$\times10^5$ CFU·g^{-1} dry soil	$\times10^3$ CFU·g^{-1} dry soil	mg CO_2·g^{-1}	mg CO_2-C·g^{-1}day^{-1}	mg C·kg^{-1}	%	mg CO_2-C· mg^{-1} Cmic· h^{-1} × 10^{-4}
Vegetable garden							
VG1	85 ±4^{a}	161 ±11^{a}	28.49 ±2.49^{a}	10.42 ±0.38^{a}	134.37 ±5.32^{a}	0.25 ±0.01^{a}	0.08 ±0.01^{a}
VG2	94 ±4^{b}	178 ±7^{b}	32.43 ±0.99^{b}	11.47 ±0.25^{a}	164.18 ±3.39^{b}	0.31 ±0.01^{ab}	0.07 ±0.00^{b}
VG3	102 ±1^{c}	191 ±2^{b}	37.72 ±0.25^{c}	13.03 ±0.82^{b}	214.66 ±8.09^{c}	0.40 ±0.01^{c}	0.06 ±0.00^{b}
VG4	434 ±1^{p}	655 ±4^{m}	59.04 ±1.57^{m}	24.18 ±0.18^{h}	504.36 ±4.53^{k}	0.62 ±0.04^{f}	0.05 ±0.00^{g}
VG5	139 ±2^{e}	243 ±10^{d}	40.75 ±0.70^{de}	14.68 ±0.29^{c}	246.79 ±4.32^{d}	0.53 ±0.00^{de}	0.06 ±0.00^{def}
VG6	272 ±10^{j}	259 ±6^{e}	47.56 ±0.15^{h}	20.25 ±0.54^{ef}	363.78 ±1.69^{fg}	0.49 ±0.00^{d}	0.06 ±0.00^{f}
VG7	155 ±2^{f}	272 ±3^{e}	43.15 ±0.56^{f}	17.08 ±0.60^{d}	291.46 ±0.96^{e}	0.48 ±0.00^{d}	0.06 ±0.00^{def}
VG8	186 ±5^{h}	291 ±12^{f}	45.72 ±0.24^{g}	21.14 ±0.49^{f}	376.45 ±3.06^{gh}	0.72 ±0.00^{ghi}	0.06 ±0.00^{ef}
Vegetable field							
VF1	176 ±11^{g}	323 ±13^{g}	45.13 ±0.23^{g}	19.77 ±0.05^{e}	354.59 ±2.49^{f}	0.69 ±0.00^{gh}	0.06 ±0.00^{f}
VF2	201 ±4^{i}	509 ±7^{h}	46.42 ±0.36^{gh}	22.67 ±0.15^{g}	394.29 ±1.59^{h}	0.73 ±0.00^{ghij}	0.06 ±0.00^{ef}
VF3	148 ±1^{f}	537 ±15^{i}	42.05 ±0.33^{ef}	24.70 ±0.30^{h}	365.19 ±5.99^{fg}	0.67 ±0.11^{fg}	0.07 ±0.00^{bc}
VF4	307 ±2^{l}	583 ±9^{j}	48.05 ±0.30^{hi}	27.85 ±0.41^{i}	457.26 ±19.78^{j}	0.81 ±0.04^{k}	0.06 ±0.00^{def}
VF5	285 ±10^{k}	606 ±6^{k}	49.57 ±0.47^{i}	27.43 ±0.51^{i}	430.43 ±5.10^{i}	0.75 ±0.01^{hij}	0.06 ±0.00^{cd}
VF6	362 ±7^{n}	607 ±2^{k}	51.41 ±1.09^{j}	28.40 ±1.15^{i}	462.66 ±5.14^{j}	0.77 ±0.01^{ijk}	0.06 ±0.00^{ed}
Paddy field sites							
P1	339 ±11^{m}	684 ±9^{n}	53.40 ±0.69^{k}	24.00 ±0.90^{h}	413.72 ±4.96^{i}	0.56 ±0.02^{e}	0.06 ±0.00^{ef}
P2	380 ±4^{o}	513 ±5^{h}	55.20 ±1.14^{l}	32.33 ±1.04^{j}	562.98 ±5.01^{l}	0.79 ±0.01^{jk}	0.06 ±0.00^{ef}
P3	114 ±1^{d}	215 ±11^{c}	39.28 ±0.51^{cd}	16.67 ±1.04^{d}	221.22 ±9.29^{c}	0.34 ±0.02^{bc}	0.08 ±0.01^{a}
P4	439 ±2^{p}	622 ±6^{l}	68.45 ±0.57^{n}	28.43 ±0.60^{i}	567.40 ±40.03^{l}	0.69 ±0.05^{gh}	0.05 ±0.00^{g}
Reference site							
C	448 ±0.1^{q}	686 ±4^{n}	72.25 ±1.61^{o}	42.17 ±1.76^{k}	982.67 ±6.00^{m}	1.12 ±0.02^{l}	0.04 ±0.00^{h}
Vegetable garden	183 ±113^{x}	281 ±151^{x}	41.86 ±9.12 ^{x}	16.53 ±4.76^{x}	287.01 ±117.86^{x}	0.47 ±0.01^{x}	0.06 ±0.01^{x}
Vegetable field	246 ±79^{xy}	527 ±101^{y}	47.11 ±3.16 ^{xy}	25.14 ±3.24^{y}	410.74 ±44.20^{y}	0.74 ±0.00^{y}	0.06 ±0.00^{x}
Paddy field	318 ±128^{y}	509 ±188^{y}	54.08 ±10.81^{y}	25.36 ±6.13^{y}	441.33 ±148.69^{y}	0.59 ±0.01^{x}	0.06 ±0.01^{x}
Reference	448 ±1^{z}	686 ±4^{z}	72.25 ±1.61^{z}	42.17 ±1.76^{z}	982.67 ±6.00^{z}	1.12 ±0.00^{z}	0.04 ±0.00^{y}

Each mean is the average of the values obtained for three samples of each soil. Values in the same column followed by the same letter(s) are not significantly different at $p<0.05$ according to ANOVA. Sampling sites legend description in Table 2.

number and activities in soils with high heavy metal content (Table 7). The *q*Mic decreased with increasing the soil heavy metal content. The qCO_2 varied widely and showed an increasing trend with the decrease of heavy metal content. The mechanism involved in inactivating and inhibiting soil microbial activity differs for different heavy metals. In our study, soil microbiological properties showed considerable differences relating to different heavy metals; the negative effects from heavy metal to soil microbial properties have been reported, suggesting that soil microbial properties are significantly inhibited by heavy metals (Li et al., 2018). A marked decrease in total cultivable numbers of soil microorganisms for the heavy metal contaminated soil samples indicate that Pb, Cd, Zn and Cu inhibit soil microbial population (Abdu et al., 2017). Bacteria seem to be more sensitive to heavy metal contamination than fungi (Table 7). According to Liu et al., (2007), the way heavy metals act depends on their type and rate. Lead doses above 50 mg·kg^{-1} decreased the count of both bacteria and fungi. Khan et al., (2008) showed the inhibitory effect of high Cd and Pb concentrations on soil MBC. Heavy metals decrease MBC and reduce their activity in the soil (Wyszkowska et al., 2008). MBC is a sensitive parameter and can be used as an indicator of changes in OM composition (Yang et al., 2006). The decrease in MBC caused by a high level of heavy metal contamination found at the sites agrees with Wang et al., (2007). The

a

	pH	OM	TN	AvP	Clay	Cd	Cr	Cu	Ni	Pb	Zn
pH	1.00	0.83	0.79	0.79	0.90	-0.85	-0.90	-0.84	-0.83	-0.83	-0.88
OM	0.83	1.00	0.69	0.61	0.66	-0.63	-0.74	-0.60	-0.59	-0.60	-0.68
TN	0.79	0.69	1.00	0.71	0.57	-0.73	-0.78	-0.70	-0.67	-0.72	-0.80
AvP	0.79	0.61	0.71	1.00	0.72	-0.87	-0.84	-0.85	-0.83	-0.86	-0.84
Clay	0.90	0.66	0.57	0.72	1.00	-0.75	-0.73	-0.77	-0.78	-0.77	-0.76
Cd	-0.85	-0.63	-0.73	-0.87	-0.75	1.00	0.95	0.98	0.96	0.97	0.96
Cr	-0.90	-0.74	-0.78	-0.84	-0.73	0.95	1.00	0.93	0.90	0.93	0.96
Cu	-0.84	-0.60	-0.70	-0.85	-0.77	0.98	0.93	1.00	0.99	0.99	0.96
Ni	-0.83	-0.59	-0.67	-0.83	-0.78	0.96	0.90	0.99	1.00	0.97	0.92
Pb	-0.83	-0.60	-0.72	-0.86	-0.77	0.97	0.93	0.99	0.97	1.00	0.96
Zn	-0.88	-0.68	-0.80	-0.84	-0.76	0.96	0.96	0.96	0.92	0.96	1.00

b

	TLM	DH	URE	AP	AS	BAC	FUN	SIR	MA	MBC	*q* Mic	*q* CO_2
TLM	1.00	-0.87	-0.96	-0.78	-0.84	-0.89	-0.83	-0.91	-0.88	-0.86	0.78	-0.84
DH	-0.87	1.00	0.93	0.95	0.99	0.96	0.82	0.97	0.88	0.93	-0.74	0.75
URE	-0.96	0.93	1.00	0.86	0.91	0.95	0.86	0.96	0.88	0.88	-0.78	0.79
AP	-0.78	0.95	0.86	1.00	0.96	0.86	0.73	0.95	0.82	0.92	-0.74	0.71
AS	-0.84	0.99	0.91	0.96	1.00	0.93	0.79	0.96	0.87	0.95	-0.75	0.75
BAC	-0.89	0.96	0.95	0.86	0.93	1.00	0.85	0.93	0.85	0.85	-0.71	0.70
FUN	-0.83	0.82	0.86	0.73	0.79	0.85	1.00	0.81	0.85	0.77	-0.53	0.76
SIR	-0.91	0.97	0.96	0.95	0.96	0.93	0.81	1.00	0.88	0.92	-0.81	0.77
MA	-0.88	0.88	0.88	0.82	0.87	0.85	0.85	0.88	1.00	0.95	-0.60	0.91
MBC	-0.86	0.93	0.88	0.92	0.95	0.85	0.77	0.92	0.95	1.00	-0.75	0.89
q Mic	0.78	-0.74	-0.78	-0.74	-0.75	-0.71	-0.53	-0.81	-0.60	-0.75	1.00	-0.67
q CO_2	-0.84	0.75	0.79	0.71	0.75	0.70	0.76	0.77	0.91	0.89	-0.67	1.00

Fig. 5. Pearson's correlation analysis correlating (a) soil physicochemical properties and heavy metals (b) TLM, soil microbiological properties and enzyme activities (n = 56) ($p < 0.05$). Green boxes show positive correlations; red boxes show negative correlations. Explanations: pH = Soil reaction, OM = Soil organic matter, TN = Total nitrogen, AvP = Available phosphorus, Clay = Clay content, Cd = Cadmium, Cr = Chromium, Cu = Cupper, Ni = Nickel, Pb = Lead, Zn = Zinc, TLM = Total load of extractable metals, DH = Dehydrogenase, URE= Urease, AP = Acid phosphatase, AS = Arylsulfatase, BAC = The number of cultivable bacteria, FUN = The number of cultivable fungi, SIR=Substrate induced respiration, MA = Microbial activity, MBC = Microbial biomass carbon, *q*Mic = Microbial quotient, *q*CO_2= Metabolic quotient

Table 7
Correlation coefficients among heavy metals and soil microbial and enzymatic properties

Variables		Equations	R^2	*r*
Dependent	Independent			
Cadmium	Bacteria	y = –55.98x + 441.68	0.75	–0.86
	Fungi	y = –81.46x + 713.58	0.66	–0.81
	SIR	y = –5.12x + 65.62	0.83	–0.91
	MA	y = –3.55x + 34.90	0.77	–0.88
	MBC	y = –83.54x + 687.82	0.74	–0.86
	Dehydrogenases	y = –84.61x + 708.03	0.74	–0.86
	Urease	y = –5.60x + 53.77	0.90	–0.95
	Acid phosphatase	y = –13.45x + 166.35	0.60	–0.77
	Arylsulfatase	y = –12.13x + 82.94	0.68	–0.82
Chromium	Bacteria	y = –2.10x + 576.77	0.84	–0.92
	Fungi	y = –2.94x + 891.72	0.69	–0.83
	SIR	y = –0.19x + 77.88	0.93	–0.96
	MA	y = –0.13x + 43.45	0.87	–0.93
	MBC	y = –3.29x + 912.42	0.91	–0.95
	Dehydrogenases	y = –3.31x + 933.50	0.90	–0.95
	Urease	y = –0.20x + 65.74	0.92	–0.96
	Acid phosphatase	y = –0.56x + 206.66	0.81	–0.90
	Arylsulfatase	y = –0.49x + 117.54	0.88	–0.94
Cupper	Bacteria	y = –0.64x + 397.65	0.75	–0.87
	Fungi	y = –0.94x + 649.56	0.66	–0.81
	SIR	y = –0.06x + 61.02	0.77	–0.88
	MA	y = –0.04x + 31.76	0.72	–0.85
	MBC	y = –0.90x + 608.49	0.66	–0.81
	Dehydrogenases	y = –0.94x + 633.62	0.69	–0.83
	Urease	y = –0.06x + 49.22	0.89	–0.94
	Acid phosphatase	y = –0.14x + 152.93	0.51	–0.72
	Arylsulfatase	y = –0.13x + 71.81	0.62	–0.79
Nickle	Bacteria	y = –1.75x + 395.67	0.77	–0.88
	Fungi	y = –2.51x + 644.06	0.67	–0.82
	SIR	y = –0.15x + 60.32	0.73	–0.85
	MA	y = –0.10x + 31.18	0.67	–0.82
	MBC	y = –2.30x + 592.45	0.59	–0.77
	Dehydrogenases	y = –2.49x + 625.61	0.68	–0.82
	Urease	y = –0.17x + 48.68	0.87	–0.93
	Acid phosphatase	y = –0.36x + 150.34	0.46	–0.68
	Arylsulfatase	y = –0.35x + 70.31	0.59	–0.77
Lead	Bacteria	y = –0.28x + 387.52	0.74	–0.86
	Fungi	y = –0.42x + 641.19	0.69	–0.83
	SIR	y = –0.03x + 60.31	0.77	–0.88
	MA	y = –0.02x + 31.29	0.73	–0.86
	MBC	y = –0.40x + 597.84	0.67	–0.82
	Dehydrogenases	y = –0.41x + 619.57	0.68	–0.83
	Urease	y = –0.03x + 48.47	0.90	–0.95
	Acid phosphatase	y = –0.06x + 151.67	0.53	–0.73
	Arylsulfatase	y = –0.06x + 70.04	0.62	–0.79
Zinc	Bacteria	y = –0.37x + 467.14	0.79	–0.89
	Fungi	y = –0.52x + 741.61	0.65	–0.81
	SIR	y = –0.03x + 68.09	0.88	–0.94
	MA	y = –0.02x + 36.37	0.80	–0.89
	MBC	y = –0.56x + 730.91	0.80	–0.89
	Dehydrogenases	y = –0.56x + 750.17	0.79	–0.89
	Urease	y = –0.04x + 55.85	0.91	–0.95
	Acid phosphatase	y = –0.09x + 175.35	0.70	–0.84
	Arylsulfatase	y = –0.08x + 89.83	0.75	–0.87

All data are significant at a confidence interval of 95%. Legend description in Fig. 5.

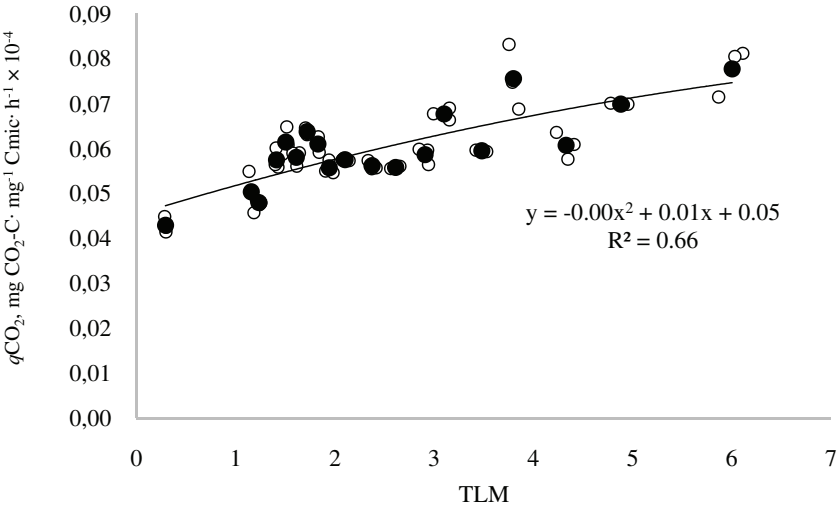

Fig. 6. Relationship of metabolic quotient (qCO_2) with total load of extractable metals (TLM) in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram. Regression equation, line of the best fit and R^2 is shown. Filled circles representing the average qCO_2 values and open circles representing the replications

inhibition of MBC in soils highly contaminated by heavy metals (Wyszkowska et al., 2013). The MA, apart from reflecting the rate of mineralization of soil OM, reflects the respiratory activity of soil microorganisms, which is closely related to soil environmental quality (Schloter et al., 2018). Heavy metals may reduce soil MA by forming complexes with the substrates or by killing sensitive microorganisms (Landi et al., 2000). The synthesis of MBC in soils polluted by heavy metals can be less effective than in non-polluted soils due to the stress caused by heavy metals. Yang et al., (2006) stated that Cd, Pb and Zn cause disorders in the soil MA and depress the MBC of microorganisms.

The *q*Mic has been proposed as a useful measure of soil pollution of heavy metals (Wang et al., 2007). Our results confirm these findings, because the *q*Mic values, expressing the maintenance energy, as the amount of heavy metal in soil increased (Table 6). Šmejkalová et al., (2003) was also found a significant decline of the *q*Mic with an increasing level of contamination. Soil microorganisms can be adapted to long-term heavy metal pollution by several mechanisms, such as precipitation of metals as phosphates, carbonates and sulfides, physical exclusion by exopolymers, and intracellular sequestration with low molecular weight compounds (Wang et al., 2007). This kind of cellular activity requires huge energy that increases the demand for maintenance energy. To survive in stress, soil microorganisms reduce the conversion of substrate into new MBC and other metabolic processes, therefore *q*Mic decreased. A reduction of this ratio as a result of metal pollution has been reported from other studies (Valentim dos Santos et al., 2016). *q*Mic also shows the survival capacity of soil microorganisms. Soil pollution due to heavy metal contamination is a serious problem because tolerant microorganisms can bioaccumulate heavy metals that directly affect the food chain to human health (Zhuang et al., 2009). On the other hand, soil microorganisms under environmental stress shift more energies from growth and reproduction to maintenance and survival, leading to an increase in qCO_2 (Zhao et al., 2020). Our results show that the qCO_2 increased markedly with increasing heavy metal concentration (Table 6). Zhang et al., (2008) also found the qCO_2 as a good indicator of the negative impact of heavy metal pollution on soil microorganisms. A correlation study also demonstrated that qCO_2 was negatively correlated with soil MBC, microbial number and activity (Fig. 5) but qCO_2 was significantly positively correlated with total heavy metals (TLM) (Fig. 6).

3.3. Effect of heavy metals on soil enzyme activity

The dehydrogenases, urease, acid phosphatase and arylsulfatase, enzymes involved in the C-N-P-S cycle in soil varied widely among the soils studied. The results demonstrated that the enzymatic activities of soil were lower than the reference site. The level of enzyme activity varied in a wide range and for dehydrogenases amounted 187.09 to 729.17 mg formazan·kg $soil^{-1}$ $24h^{-1}$, for urease 13.48 to 50.22 mg NH_4-N·kg soil· $2h^{-1}$, for acid phosphatase 82.12 to 183.52 mg p-nitrophenol·kg $soil^{-1}$ h^{-1}, and arylsulfatase 10.60 to 80.65 mg p-nitrophenol· kg $soil^{-1}$ h^{-1}. The average enzyme activity is presented in Table 8. On the reference site mean concentration of dehydrogenases, urease, acid phosphatase and arylsulfatase were 878.09 mg formazan·kg $soil^{-1}$ $24h^{-1}$, 53.06 mg NH_4-N·kg soil· $2h^{-1}$, 217.46 mg p-nitrophenol·kg soil $^{-1}$ h^{-1}, and 119.11 mg p-nitrophenol·kg $soil^{-1}$ h^{-1} respectively. Enzymatic activities were significantly ($p < 0.05$) low in the vegetable garden, vegetable field, paddy fields soils than in the reference site. A significant decrease in all the four enzyme activities was observed in the samples of agricultural soils near acid – leaching, burning and dismantling sites compared with the reference site (C). A significant positive correlation ($p < 0.05$) between dehydrogenases, urease, acid phosphatase and arylsulfatase was found in this study (Fig. 5).

Soil enzymes are biologically active soil components that have an intimate association with physicochemical and biological soil characteristics (Shukla and Varma, 2011). Soil enzyme activity is widely used as a reliable biological indicator to assess soil contamination but no quantitative standard of soil enzyme activity has been set to assess the level of heavy metal soil pollution. Generally, high enzyme activity represents good soil quality, while low activity may be related to the toxicity of heavy met-

Table 8
Soil enzymes involved in soil C (dehydrogenases), N (urease), P (acid phosphatase) and S (arylsulfatase) (mean ±SD) turnover in soils in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram.

Legend	Dehydrogenases	Urease	Acid phosphatase	Arylsulfatase
	mg formazan·kg soil^{-1} 24h^{-1}	mg NH_4–N·kg soil· 2h^{-1}	mg p-nitrophenol·kg soil $^{-1}$ h^{-1}	mg p-nitrophenol· kg soil $^{-1}$ h^{-1}
Vegetable garden				
VG1	187.09 ±7.98^{a}	13.48 ±1.27^{a}	82.12 ±2.06^{a}	10.60 ±0.06^{a}
VG2	197.82 ±3.33^{ab}	14.94 ±0.15^{b}	85.84 ±2.82^{ab}	11.57 ±0.17^{ab}
VG3	217.59 ±1.71^{bc}	18.83 ±0.35^{c}	89.63 ±2.95^{b}	12.48 ±0.14^{bc}
VG4	612.61 ±37.03^{k}	48.18 ±0.68^{p}	157.27 ±7.26^{i}	74.83 ±0.70^{m}
VG5	237.87 ±2.68^{cd}	29.29 ±0.17^{e}	101.80 ±1.66^{cd}	17.13 ±0.34^{d}
VG6	408.81 ±6.47^{g}	35.80 ±1.00^{j}	108.26 ±3.28^{de}	34.49 ±0.61^{g}
VG7	280.44 ±24.79^{e}	30.94 ±0.12^{fg}	105.55 ±1.21^{d}	27.48 ±0.30^{e}
VG8	350.13 ±1.83^{f}	32.19 ±0.20^{h}	106.54 ±1.47^{d}	30.22 ±0.24^{f}
Vegetable field				
VF1	340.18 ±4.12^{f}	31.36 ±0.17^{gh}	106.24 ±0.64^{d}	28.12 ±0.46^{e}
VF2	363.36 ±2.00^{f}	33.51 ±0.53^{i}	108.26 ±0.33^{de}	33.06 ±0.10^{g}
VF3	256.34 ±2.19^{de}	30.27 ±0.17^{f}	104.26 ±0.23^{d}	17.90 ±0.35^{d}
VF4	440.30 ±6.03^{h}	36.91 ±0.23^{k}	114.78 ±2.13^{ef}	37.74 ±0.85^{h}
VF5	457.71 ±6.46^{h}	39.26 ±1.13^{l}	117.86 ±0.39^{f}	47.47 ±0.47^{i}
VF6	529.32 ±3.38^{i}	40.89 ±0.15^{m}	121.41 ±0.49^{fg}	52.00 ±0.80^{j}
Paddy field sites				
P1	543.66 ±5.09^{ji}	42.86 ±0.37^{n}	127.30 ±2.53^{gh}	55.46 ±0.66^{k}
P2	558.95 ±8.17^{j}	45.99 ±0.25^{o}	130.41 ±1.13^{h}	65.04 ±0.03^{l}
P3	231.74 ±2.49^{cd}	21.07 ±0.26^{d}	97.01 ±2.40^{c}	13.30 ±0.17^{c}
P4	729.17 ±37.08^{l}	50.22 ±0.30^{q}	183.52 ±12.23^{j}	80.65 ±0.43^{n}
Reference site				
C	878.29 ±20.72^{m}	53.06 ±0.59^{r}	217.46 ±4.42^{k}	119.11 ±3.32^{o}
Land use types				
Vegetable garden	311.54 ±138.30^{x}	27.96 ±11.21^{x}	104.63 ±22.67^{x}	27.35 ±20.33^{x}
Vegetable field	397.87 ±91.35^{xy}	35.37 ±4.09^{xy}	112.14 ±6.52^{x}	36.05 ±11.81^{xy}
Paddy field	515.88 ±188.17^{y}	40.04 ±11.76^{y}	134.56 ±32.97^{y}	53.61 ±26.06^{y}
Reference	878.29 ±20.72^{z}	53.06 ±0.59^{z}	217.46 ±4.42^{z}	119.11 ±3.32^{z}

Each mean is the average of the values obtained for three samples of each soil. Values in the same column followed by the same letter(s) are not significantly different at $p<0.05$ according to ANOVA. Sampling sites legend description in Table 2.

als pollutants to biological processes (Fazekašová and Fazekaš, 2020). Agricultural soils with high heavy metal concentrations showed reduced soil enzyme activities. The highest inhibitory effect on soil enzymes was observed in the most polluted soils. Soil enzymatic activity values were also correlated strongly and negatively with heavy metal concentrations. These findings express that if present at toxic concentration, heavy metals have a negative impact on soil enzyme activities. Cr and Zn had shown a very high significant negative correlation with the enzymes-dehydrogenases, urease, acid phosphatase and arylsulfatase (Table 7). It was also observed that urease activity was the most affected by Cr while the least by Ni. The heavy metal toxicity trend for toxicity impact for the enzyme was as follows: Cr > Cd = Zn = Pb > Cu > Ni. Cd and Cu, depress the activity of soil metals if present in excessive amounts (Kucharski et al., 2011). In turn, Speir et al., (1999) proved that Cd and Ni are stronger inhibitors than Cu and Cr. For the heavy metals (Table 7) the decreasing trend of soil enzyme activities was as urease > dehydrogenases > arylsulfatase > acid phosphatase. Results in our study showed that the urease and dehydrogenases activity were more sensitive to the heavy metal stress than the acid phosphatase activity. Zhang et al., (2013) showed dehydrogenases activity reduced in metal-contaminated soil compared to uncontaminated soil but soil phosphatase showed no response. Phosphatase activity can occur extracellularly along with within a living cell whereas dehydrogenases activity only acts inside a living cell (Wang et al., 2007). Therefore, microbial activity inhibited by heavy metal stresses directly expresses less dehydrogenases activity.

ED_{50} values can be a suitable indicator of the sensitivity of an ecosystem to stress, because a 50% reduction of a basic ecological process may be too extreme for its continued functioning. Table 9 shows the four enzyme activities measured for the heavy metal contaminated agricultural soils and ED_{50} values calculated from the best fit model and R^2 values from the regression analysis. Studies on the impact of toxic metals on soil enzymes showed the inhibition of these enzyme activities was always less than 100% of the control value. Model 1 was a full inhibition model. Model 2 was a partial inhibition model, suggesting that a fraction of the enzymatic activities were not inhibited by heavy metal contamination to the soil. Model 3 indicates that the relationship between enzyme activity and the toxicity coefficient is sigmoidal dose. It is very hard to interpret the reason for the decrease in soil enzyme activities as it may be due to a direct metal inhibition to enzymes or a lower synthesis and release of enzymes, or a combination of both (Gao et al., 2010). Enzymes in soils can be physically and chemically protected by soil constituents (organic and inorganic ligands), which interacted with trace elements (Renella et al., 2003), whereas enzyme was influenced by many factors not only heavy metal. Therefore, among the three models, Model 2 was the best fit in most of the cases. The ED_{50} values for dehydrogenases, acid phosphatase and arylsulfatase activity were predicted with Model 2, whereas the ED_{50} values for urease activity the best fit was achieved by the sigmoidal dose-response Model 3. The arylsulfatase was sensitive to the combined heavy metal effect and easily lost activity even at low heavy metal concentration. Dehydrogenases and urease activity were also sensitive to the combined heavy metal effect. The effect of heavy metal on acid phosphatase was found to be lower than the other enzymes as phosphatase activity was high heavy metal rate responsive to inhibit it.

A significant relationship between soil enzymes and *PER* as fitted by Model 2 and Model 3, also indicates an adaptation of soil microorganisms in our study area. Microorganisms differ in their sensitivity to metal toxicity and the development of metal-tolerant strains could compensate for the loss of more sensitive populations (El Baz et al., 2015). The results of many experimental studies suggest that inhibition of soil enzymes due to heavy metal contamination can be reduced over time and some microorganisms could be adapted to long-term polluted environments and thereby help enzymatic activity to recover (Fazekašová and Fazekaš, 2020). Ship scraps dismantling activities are a long-time regular practice in this zone, therefore, the microorganism in our study site might be adapted to the high heavy metal concentration. Some of the heavy metal concentration was found to be at a very high toxic level in highly contaminated soils enough to suppress complete microbial activity, but due to their adaption capacity, they survived with limited enzyme activity.

3.4. Effect of soil physicochemical properties and heavy metal interactions on soil microbial and enzyme activity

The microbial population can be reduced by heavy metals which in turn can decrease the activities of soil enzymes. Consequently, the decomposition rate of carbon, nitrogen, phosphorus and sulfur in soils would be blocked. All the four enzymes were positively correlated ($p < 0.05$) with the number of cultivable bacterial and fungal populations, SIR, MA and MBC (Fig. 5). Dehydrogenases activity was significantly high in reference soil, where the soil microbiota was also metabolically more active than in contaminated agricultural soils (Table 6 and 8). Since dehydrogenases is an intracellular enzyme involved in microbial metabolism, their lower activity in agricultural soil may be related to the smaller MBC content, but also a higher heavy metal concentration in agricultural than in reference soils. Furthermore, the dehydrogenases activity was significantly correlated with MBC ($r = 0.93$, $p < 0.05$). The decrease of soil MBC and inhibition of dehydrogenases activity have been reported in polluted areas near an aluminium smelter with water-extractable fluoride concentration over 100 $mg \cdot kg^{-1}$ in soil (Tscherko and Kandeler, 1997) These results suggest that MBC and dehydrogenases can be useful measures of the level of heavy metal contamination in a soil sample. Arylsulfatase, urease and acid phosphatase activity also showed a positive correlation with MBC ($p < 0.05$), MA and qCO_2 ($p < 0.05$).

The effects of heavy metal contamination on enzyme activities can be mediated by soil pH (Dick, 2011), OM content (Tang et al., 2020) and clay content (Tietjen and Wetzel, 2003). Soil pH is one of the very important factors that are considered to evaluate the effect of pollutants on the activity of soil microorganisms, however, it is very difficult to separate the effect of heavy metal stress on soil microbial populations from that due to pH changes (García-Gil et al., 2013). Changing soil pH to an acidic level due to the soil management effect may intensify the heavy metal effect further (Wyszkowska et al., 2016). The decreasing pH leads to the increased bioavailability of Cu, Cd, Zn and Pb in soil (Aponte et al., 2020), which results in higher heavy metal toxicity for microorganisms and inhibition of enzyme activities. The nutrient content of the soil has a regulatory effect on the toxicity of heavy metals in soil (Chodak et al., 2013). But in our findings (Table 7 and 10), the effect of OM, TN, AvP on microorganisms was not as strong as that of heavy metal content. The changing

Table 9
Values of R^2 ($p < 0.05$) obtained for Gauss-Newton analysis, which best describe the inhibition of dehydrogenases, urease, acid phosphatase and arylsulfatase of different land-use type and 50% ecological dose (ED_{50}) expressed by total ecological toxicity coefficient (*PER*)

Enzyme	Model	ED_{50}	R^2
Dehydrogenases	2	3034.26	0.89
Urease	3	204.58	0.97
Acid Phosphatase	2	3564.02	0.86
Arylsulfatase	2	461.62	0.91

Table 10
Correlation coefficients among physicochemical properties and soil microbial and enzymatic properties

Variables		Equation	R^2	*r*
Dependent	Independent			
pH	Bacteria	y = 221.31x – 867.23	0.90	0.95
	Fungi	y = 295.12x – 1055.70	0.67	0.82
	SIR	y = 19.15x – 48.62	0.90	0.95
	MA	y = 11.84x – 37.06	0.66	0.81
	MBC	y = 296.80x – 1097.20	0.72	0.85
	Dehydrogenases	y = 335.65x – 1276.00	0.89	0.94
	Urease	y = 20.04x – 66.61	0.89	0.94
	Acid phosphatase	y = 55.24x – 158.50	0.78	0.88
	Arylsulfatase	y = 49.45x – 208.18	0.87	0.93
OM	Bacteria	y = 487.76x – 282.05	0.66	0.81
	Fungi	y = 538.04x – 153.77	0.33	0.58
	SIR	y = 42.71x + 1.49	0.67	0.82
	MA	y = 23.776x – 3.26	0.40	0.64
	MBC	y = 651.93x – 310.02	0.52	0.72
	Dehydrogenases	y = 766.50x – 417.48	0.70	0.84
	Urease	y = 40.63x – 9.79	0.55	0.74
	Acid phosphatase	y = 134.65x – 26.42	0.70	0.84
	Arylsulfatase	y = 114.86x – 83.79	0.71	0.84
TN	Bacteria	y = 3175.90x – 248.03	0.57	0.75
	Fungi	y = 4349.90x – 247.83	0.45	0.67
	SIR	y = 325.96x – 2.97	0.80	0.89
	MA	y = 194.06x – 7.70	0.55	0.74
	MBC	y = 4971.50x – 377.48	0.62	0.79
	Dehydrogenases	y = 5289.40x – 410.42	0.68	0.83
	Urease	y = 303.82x – 13.058	0.63	0.79
	Acid phosphatase	y = 972.69x – 31.94	0.74	0.86
	Arylsulfatase	y = 765.50x – 78.52	0.64	0.80
AvP	Bacteria	y = 43.72x – 208.56	0.63	0.79
	Fungi	y = 72.12x – 320.81	0.72	0.85
	SIR	y = 3.87x + 7.47	0.66	0.81
	MA	y = 2.87x – 7.34	0.70	0.84
	MBC	y = 61.29x – 241.43	0.55	0.74
	Dehydrogenases	y = 63.98x – 252.81	0.58	0.76
	Urease	y = 4.27x – 10.19	0.73	0.85
	Acid phosphatase	y = 10.12x + 14.18	0.47	0.69
	Arylsulfatase	y = 8.91x – 52.13	0.51	0.71
Clay	Bacteria	y = 92.53x – 2460.10	0.67	0.82
	Fungi	y = 130.44x – 3386.00	0.55	0.74
	SIR	y = 7.33x – 166.60	0.56	0.75
	MA	y = 4.65x – 113.61	0.44	0.66
	MBC	y = 106.89x – 2730.60	0.40	0.63
	Dehydrogenases	y = 127.30x – 3310.90	0.55	0.74
	Urease	y = 8.64x – 218.37	0.70	0.84
	Acid phosphatase	y = 18.61x – 424.89	0.38	0.61
	Arylsulfatase	y = 18.54x – 501.80	0.52	0.72

All data are significant at a confidence interval of 95%. Legend description in Fig. 5.

redox potential in paddy soils can control the mobility, potential toxicity and ultimate fate of heavy metals in these soils. Consequently, the concentrations of soluble Pb, Cd, Zn and Cu from paddy fields with aerobic-anaerobic cycles can be slightly lower than those aerated with oxygen (Li et al., 2018). Besides, the non-uniform distribution of scrap processing activities related to land-use practices causes some anomalous soil properties. Paddy soil exhibited high large arylsulfatase and acid phosphatase activities, which may indicate less stress compared to vegetable garden soil due to the changing redox conditions. Moreover, at improved pH conditions from very acid to the moderately acidic situation (paddy soil), the bioavailability of heavy metals may be reduced, but the improved pH conditions may affect not only microbial numbers and activities but also soil enzyme activity (García-Gil et al., 2013).

3.5. Effect of total load of extractable heavy metals (TLM) on soil microbial and enzyme activity

All the heavy metals measured from a certain site together can be used as TLM to predict the toxicity of heavy metals of that site to the soil microorganisms, which would explain the influence of combined pollution on soil microbial activity. Furthermore, compared with one single metal, multiple heavy metals in soil behave interactively and show combined ecological influence in nature. Heavy metals in combined impact can have synergistic or antagonistic effects on soil enzymes as they influence the absorption, distribution and usage of each other. Heavy metals can have different inhibiting orders on enzyme activities (Gülser and Erdoğan, 2008). The results of this study found suppression of all the soil microbial properties and enzyme activities, which indicated the disruption of soil function by the heavy metal contamination in the vicinity of ship scrap processing sites. The qCO_2, which expresses the stress situation on soil microorganisms, with increasing TLM was fitted by the exponential curve (Fig. 6). Correlation analysis produced significant relationships between TLM versus all the microbial properties and enzyme activities (Fig. 5). All of the measured parameters showed a significant decline with increasing TLM and the reduction was particularly evident at the highest TLM values (sites VG1, VG2, VG3, P3, VG5 and VF3). The inhibitory effect of Cd and Pb on the urease, acid phosphatase and dehydrogenases enzyme activity was greater when combined than single heavy metal (Pan and Yu, 2011), this was also supported by Cd, Pb and Zn combination (Yang et al., 2006). Whereas Cu toxicity was greater as single metal than in combination with Cd, Cr, Ni, Pb and Zn (Wyszkowska et al., 2006). Therefore, heavy metal type and concentration in their combined action influence the synergistic or antagonistic effect of heavy metals on soil microbial and enzyme activity.

3.6. Characterization of agricultural soils around ship scrap processing sites

A PCA was performed on a correlation matrix of the data obtained on soil microbial and enzymatic activities affected by soil physicochemical properties and heavy metal content (Fig. 7). The correlation circle revealed a strong relationship

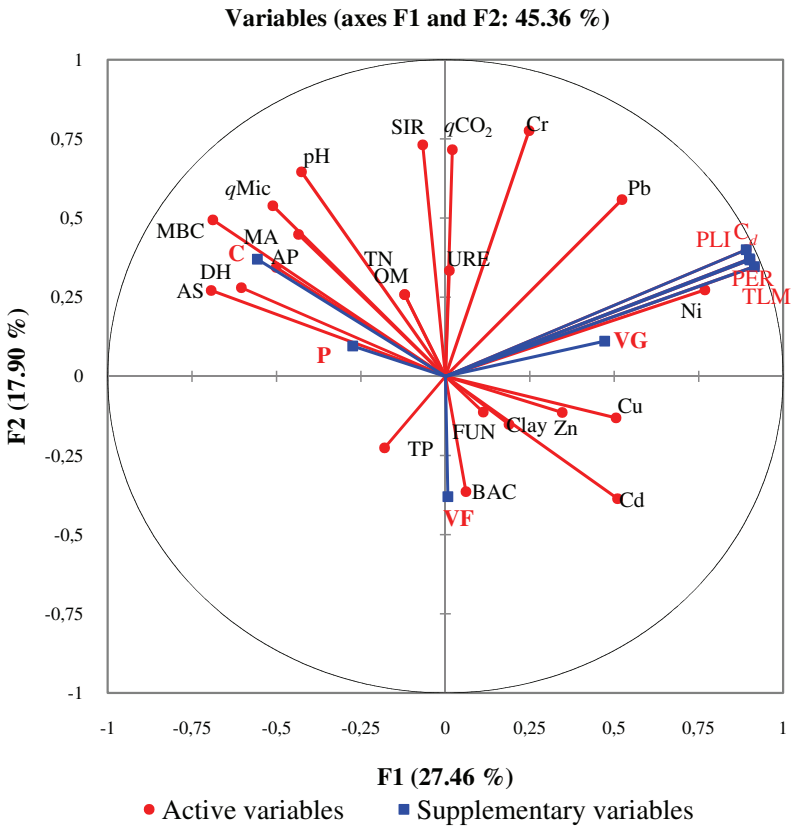

Fig. 7. Results of principal component analysis (PCA) of soil microbial activity and enzyme activity in soil contaminated with heavy metals in different agricultural soils besides ship scrap processing sites, Sitakunda, Chattogram. Explanations: pH = Soil reaction, OM = Soil organic matter, TN = Total nitrogen, AvP = Available phosphorus, Clay = = Clay content, Cd = Cadmium, Cr = Chromium, Cu = Cupper, Ni = Nickel, Pb = Lead, Zn = Zinc, TLM = Total load of extractable metals, DH = Dehydrogenase, URE = Urease, AP = Acid Phosphatase, AS = Arylphophatase, BAC = The number of cultivable bacteria, FUN = The number of cultivable fungi, SIR = Substrate induced respiration, MA = Microbial activity, MBC = Microbial biomass carbon, *q*Mic = Microbial quotient, qCO_2 = Metabolic quotient, *PLI* = Pollution load index, C_d = Integrated pollution degree, *PER* = Potential ecological risk, TLM = Total load of extractable metals, VG = Vegetable garden site, VF = Vegetable field site, P = Paddy field site, C = Reference site

between enzymatic activities and soil microbial properties, and they varied together in the same trend upon toxic impact from heavy metals. The PCA analysis showed that microbial biomass and activities (MA and SIR) and all the enzyme activities were highly associated with soil pH, OM and TN. There was a very close association between all the heavy metal indices. Cr, Pb, Ni and Cu, Cd and Zn were significantly and positively associated with all the indices of heavy metal and qCO_2. Dehydrogenases, acid phosphatase and arylsulfatase responded similarly to soil contamination with heavy metals, which is demonstrated by the proximity of vectors representing the analyzed enzymes. Urease was also sensitive to heavy metals, but its response to heavy metals was somewhat different. This is illustrated by the position of the urease vector relative to cases representing the soil heavy metal indices (TLM, *PER*, C_d and *PLI*). The distribution of sampling area (VG, VF, P and C) in the PCA plot also manifests the difference between them. Gao et al., (2010) showed that absolute enzymatic activities varied under different land uses depending on the types of land use or management and the type of enzyme.

Dendrogram grouping of heavy metal contaminated soils characterized by similar responses of soil microorganisms and their enzyme activity to heavy metal concentration and respective heavy metal indices along with soil physicochemical properties were performed (Fig. 8). The dendrogram revealed two main clusters of similarities with heavy metal contaminated soils. A cluster that contained P4, VG4 and C was significantly different from the other agricultural soils. As P4 and VG4 are clustered together with reference site, we can tell that the environmental situation in these soils with a combination of multiple heavy metals with physicochemical characteristics of the soils, were not under any stressed condition. Among the contaminated sites, there were two clusters and a cluster that contained only one element VG1 was significantly different from the other contaminated sites. The soil of this site is at the highest pollution level as supported by heavy metal indices and the related responses of microbial and enzyme activities.

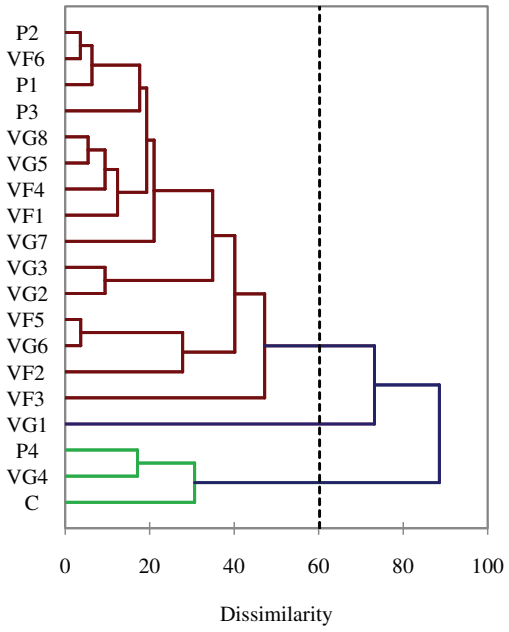

Fig. 8. Similarity dendrogram for sampling sites. Sampling sites legend description in Table 2

4. Conclusions

Investigation on the heavy metal concentration and heavy metal indices from the agricultural soils in the vicinity of ship scrap processing activities showed that:

- the heavy metal contents of the soil were at a high pollution level,
- the physicochemical properties especially the pH of these soils played an important role in enhancing the bioavailability of heavy metals,
- at the current pollution level, the heavy metals in the soil of the shipbreaking area affect the soil quality as they exhibit a significant inhibitory effect on the soil microbial and enzyme activity,
- due to the emerging environmental issues of heavy metal contamination in agricultural soils, high amounts of multiple heavy metal pollutants in contaminated soil need further study to confirm the enzyme kinetics and mechanisms for the effects of heavy metal interaction,
- the area under the impact of shipbreaking is becoming wide-spread in Chattogram and includes major cropping and forested zones in coastal areas. Further studies on the possibility of toxic effect mechanism for heavy metal in soil enzyme and microbiological activities and translocation to vegetable plants growing in the area are necessary,
- the results obtained in this study are alarming and the Government authorities of Chattogram in Bangladesh should undertake appropriate strategies to establish rules of safe working near shipyards for people and protecting the environment from heavy metal contaminations.

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