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RESEARCH ARTICLE

EFFECTS OF HEAVY METALS (Cd, Zn and Cu) ON CARBON, NITROGEN AND IRON MINERALIZATION IN SOIL

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ABSTRACT

A sixty days laboratory incubation study was conducted to investigate the effect of heavy metals on soil carbon, nitrogen and iron mineralization under aerobic condition. Sulphate salts of cadmium, zinc and copper were added individually and in combinations to soil samples and incubated in different plastic pots. Soil organic carbon did not change significantly throughout the incubation period. Soil microbial biomass carbon declined from 0.38mgkg^{-1}to 0.19 mgkg^{-1} in Cd treated soil and 0.39 mgkg^{-1} to 0.28 mgkg^{-1} in Cu treated soil which account for about 50% and 28% reduction ($p \leq 0.05$) in biomass carbon respectively. Cd:Zn and Cu:Cd treated soil had reduced 36.84% while Zn:Cu had 42.11% reduction in biomass carbon.CO_2-C effluxes peaked by day 15 for all the single metal amended soil indicating that priming effects might have occurred. But in combination, metal showed some interaction for what the respiration rates were declined for the first 15 days. Rapid ammonification with presumed immobilization took place up to day 30. The result indicated a significant ($p \leq 0.05$) net mineralization of nitrogen for Cd:Zn (63.72%) and Cu:Cd (66.66%) treatments at the end of the experiment. Available iron content showed significant changes in combined metal treatment than a single metal.

KEYWORDS

Heavy metals, microbial biomass carbon, respiration, nitrogen, iron, mineralization.

1. INTRODUCTION

Heavy metals are continuously accumulated in soil due to land disposal of municipal and industrial wastes, emissions of automobile, use of chemical fertilizers, pesticides and other toxic elements for agricultural purposes [1-4]. It is clear that heavy metals commenced with compost or sewage sludge, caused soil organic matter accumulation and decreased the turnover rate of organic matter [5-8]. Fertilizer applications possibly capable of influence Cd in soil which affects the movement of Cd to plant roots in addition to Cd uptake [9]. Copper is mainly toxic to roots. High copper interaction with iron metabolism is the causes of chlorosis as the symptom of copper toxicity [10]. Along with this, higher concentrations of copper can reduce the availability of zinc absorption to plant as these micronutrients compete for the same sites of plant root [11]. Enormously slight concentrations of some metals like copper, zinc, nickel, cadmium is vital for the components of enzymes, pigments, structural proteins, and in maintaining the ionic balance of cells [12]. Researchers all over the world are concerned about heavy metals mainly due to their harmful effects on plants, especially those on vegetative and generative parts of the plants. They also endanger human health when the metals migrate through the food chain [13]. Besides, heavy metals showed toxic effects on microorganisms in various ways such as reduced litter decomposition and nitrogen fixation, less efficient nutrient cycling [14]. There is now considerable amount of evidence of documenting decrease in the soil microbial biomass as a result of long-term exposure to heavy metal contamination from past applications of sewage sludge as reviewed by [15]. The soil organic carbon content had noteworthy positive correlations with the dehydrogenase activity, basal soil respiration, catalase activity, and microbial biomass-C at $P<0.01$ [16]. A previous researcher observed negative relationship of basal soil respiration with heavy metal contents [17]. Being a microbial dependent process, all those factors, which affect microbial activities, indirectly affect N mineralization. Several studies have shown that metals affect N mineralization, however, results are contradictory. A researcher reported that mineralization of soil organic N, as with microbial respiration, is unaffected at soil metal concentration at around current EU limits [18]. This is supported by a previous researcher who reported that inhibition of both N mineralization and nitrification are inhibited at around 1000 mgkg^{-1} Zn, Cu and Ni, around 100-500 mgkg^{-1} Pb and Cr and around 10-100 mgkg^{-1} Cd. Iron deficiency is extremely rare in the field crops [19]. As a result, very few works deal with the iron as a major nutrient that affects plant growth. Therefore, the present study has been conducted to determine the effects of heavy metals (Cd, Zn and Cu) on carbon, nitrogen and iron mineralization of an agricultural soil artificially polluted with heavy metals. For this purpose, the rates of soil organic carbon, nitrogen and iron mineralization, soil respiration and microbial biomass carbon were monitored.

2. MATERIALS AND METHODS

Agricultural clayey loamy soil samples were collected from a field plot at Noapara in Jashore district. The surface soil samples were collected from specific locations (N 23º 11.841′E: 89º 14.660′) at a depth of 0-15 cm and were processed for the subsequent experiment and analysis. The soils were air dried and ground to pass through a 2 mm sieve, sorted to remove stones, plant debris and any visible soil fauna and then were mixed thoroughly with hand trowel. The general description of the soil used for the experiment is shown in table 1.

Table 1: Characteristics of the soil under study

Properties	Analysis
pH	7.30
Carbon (%)	1.56
Total nitrogen (%)	0.11
Available nitrogen (mgkg-1)	25
Biomass carbon(mgkg-1)	0.38
Soil respiration(mg of Cg-1soil day-1)	5.64
Field capacity (%)	60.62
Texture	Clay loam
CEC (cmolckg-1)	37.60
Total Fe (%)	3.75
Available Fe (mgkg-1)	1.91

Approximately 0.25 kg of sieved soil samples were distributed into plastic pots. Solutions of analytical grade sulphate (SO_4) salt each of cadmium, zinc and copper was applied to the respective pots singly and in combinations. Soil sample which receive no metal amendment was served as control. All the treatments (A-G) were replicated three times. Number of treatments and their combinations were as follows:

Table 2: Pattern of metal amendment in pre-incubated soil pots

Treatment Notation	Metal	Concentration (mgkg^{-1})
0	Control	--
A	Cu	3000
B	Cd	3000
C	Zn	3000
D	Cu:Cd	1500:1500
E	Cd:Zn	1500:1500
F	Zn:Cu	1500:1500
G	Cu:Cd:Zn	1000:1000:1000

Total length of the incubation period was 60 days and the time interval for collection of incubated soil sample was 0, 15, 30, 45 and 60 days. The experiment was conducted aerobically. Soil pH was determined electrochemically at soil: water ratio of 1:2.5 by using Griffin (Model 40) glass electrode pH meter as described by [20]. Organic carbon of the soil sample was determined by Walkly and Black wet oxidation method as suggested by [20]. Available nitrogen and total nitrogen of the soil both were determined by colorimetric method as suggested by [21]. Soil microbial biomass carbon was determined by fumigation extraction method as outlined by [22]. Total iron was determined by spectrophotometer after digestion with HNO_3: $HClO_4$ (2:1) mixture. Available iron was determined by 1N NH_4OAc (pH 7.0) as described by [23]. Total metal content of soil before incubation was determined after acid digestion (HNO_3:$HClO_4$; 2:1) by atomic absorption spectrophotometer. Soil respiration was done on 20g portion of soil incubated in a desiccator for 24 hours along with 5ml 0.1M NaOH followed by titration with 0.1M HCl as described in [24].

The results were analyzed by two-way analysis of variance (ANOVA) at 95% confidence interval. Differences between the control and other treatments were assessed using least significant difference (LSD) [25].

Table 3: Metal of soil detected prior to amendment as determined by atomic absorption spectrophotometer

Metal	Concentration (mgkg^{-1} soil)
Cu	0.38
Cd	0.03
Zn	3.76
Pb	0.33

3. RESULTS AND DISCUSSION

Table 4: Effects of heavy metals on changes of organic carbon in soil (%)

Treatment Days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	0.89	0.90	0.89	0.89	0.90	0.89	0.90	0.89
15	0.89	0.90	0.89	0.88	0.90	0.88	0.88	0.89
30	0.88	0.88	0.89	0.88	0.90	0.87	0.88	0.89
45	0.88	0.87	0.90	0.86	0.89	0.87	0.86	0.87
60	0.87	0.85	0.90	0.85	0.89	0.85	0.84	0.87

Organic carbon did not change significantly due to the application of heavy metals during the entire incubation period (Table 4). Among the treatments, Zn accumulated a little amount of carbon until 45 days while Cu: Cd decreased more carbon than any other treatments. From the study, it was observed that soil organic carbon was unaffected due to heavy metals application. Less incubation time may be the reason for not getting significant changes in the soil organic carbon content. A group of researchers has worked on effects of heavy metals in acid and calcareous soils for mineralization of organic carbon in incubation and found low mineralization rate of organic-C after 28 weeks of incubation [26].

Table 5: Effects of heavy metals on changes of microbial biomass carbon in soil (mg kg^{-1})

Treatment Days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	0.38	0.38	0.39	0.39	0.38	0.38	0.38	0.38
15	0.38	0.35	0.39	0.35	0.36	0.36	0.36	0.38
30	0.38	0.30	0.37	0.33	0.31	0.34	0.31	0.38
45	0.38	0.24*	0.37	0.31	0.30	0.30	0.32	0.38
60	0.37	0.19*	0.37	0.28*	0.24*	0.22*	0.24*	0.37

*Asterisks indicate significant difference from the control treatment (N=8, P≤0.05).

The table 5 showed that microbial biomass carbon (MBC) declined due to the application of heavy metals with incubation time. Cd has decreased more than any other treatments. The toxic effects of metals imposed on soil in the following order: Cd>Cu>Zn. This order probably explains the toxicity experienced by the soil microbes by the data on soil microbial biomass carbon. With respect to the MBC the result indicates that due to application of different metals and their combinations, the MBC didn't show any significant responses until day 45 except Cd. Cd, Cu itself and their combinations along with Zn showed significant (p≤0.05) decreases in soil MBC at day 60 in spite of the unusually very high levels of other metals [27]. The availability and toxicity of a given metal ion is always much lower in soil than in water solution as a result of adsorption of the metal ions by soil organic and inorganic colloids and microorganisms can overcome from their adverse effects. Interactions of metals used in combination may be the result of simple competition, antagonism, additive action or synergism [28, 29]. The finding is in agreement with a scholar who observed diminished microbial biomass C in cadmium-contaminated sewage sludge compost to the soil [30].

Table 6: Effects of heavy metals on changes of respiration in soil (mg C g^{-1} soil day^{-1})

Treatment Days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	5.64	5.40	6.82	4.70	6.30	5.20	6.20	5.90
15	9.20	8.52*	7.98	8.85*	6.40	5.50*	5.29*	5.72
30	9.15	7.90	7.39	8.24	6.01	5.51	5.18	5.73
45	8.40	7.80	7.38	7.87	5.88*	5.30	5.91	5.55*
60	7.26	7.50	7.38	7.44	5.88	5.40	4.68	4.02

*Asterisks indicate significant difference from the control treatment (N=8, P≤0.05).

Initially respiration was increased and then became slowing due to application of heavy metals during the entire incubation period (Table 6). The maximum values for respiration were found from day 0 to day 15. The result indicated that organic carbon release occurred at this time, showing the priming effect of the added organic materials. This finding is an agreement with the study of [31]. It was also found that soil respiration and especially the respiration per unit biomass (qCO2) increased with increasing amounts of heavy metals due to the contribution of fungi to soil respiration increased [32].

Table 7: Effects of heavy metals on changes of available N in soil(mg kg^{-1})

Sample	A	B	C	D	E	F	G	H
Treatment days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	82.02	86.12	95.14	61.52	83.66	77.92	66.44	83.66
15	93.16	71.36*	82.84	49.21*	58.23*	65.62*	54.92*	63.98*
30	118.11	127.13	125.49	117.29	113.19	107.45	108.27	101.71*
45	91.04	93.50	100.07	104.17	87.76	63.98*	104.17	77.10
60	46.75	46.75	40.19	41.83	30.35*	43.47	22.15*	44.29

*Asterisks indicate significant difference from the control treatment (N=8, P≤0.05).

Net N mineralization over the 60-day incubation period differed significantly (p≤0.05) among the treatments (Table 7). Minearalization and immobilization of N were closely associated in all treatments and were predominant until day 15 of the incubation period. This finding is in agreement with [33]. The highest rate for Cd (127.13mg kg^{-1}) displayed a high degree of ammonification and also for Zn (125.49 mg kg^{-1}) and Cu (117.29 mg kg^{-1}). The probable reason for the decrement of available N in soil after 30 days of incubation was due to ammonia volatilization and/or immobilization during large heterotrophic microbial activities which result in similar or lesser N to be mineralized in other treatments. Besides this, the recalcitrant organic fractions might slow down N mineralization over time. A scholar worked on nitrous oxide production from an ultisol treated with different nitrogen sources and moisture regimes and found similar results [34].

Table 8: Effects of heavy metals on changes of total N in soil (%)

Sample	A	B	C	D	E	F	G	H
Treatment days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	0.46	0.53	0.45	0.45	0.49	0.40	0.34	0.41
15	0.46	0.40	0.39	0.40	0.40	0.26*	0.29*	0.33*
30	0.46	0.44	0.38	0.44	0.52	0.46	0.50	0.43
45	0.38	0.20*	0.36	0.37	0.48	0.32	0.46	0.39
60	0.42	0.19	0.21*	0.22*	0.25*	0.21*	0.22*	0.21*

*Asterisks indicate significant difference from the control treatment (N=8, P≤0.05).

Results indicated that net N mineralization was significantly *(P≤0.05)* different among the treatments over the 60-day incubation period (Table 8). The total nitrogen content decreased throughout the incubation period. It indicates mineralization and immobilization of N were closely associated in all soils and were predominant until day 15 of the incubation period. These findings are in agreement with [34]. The highest value for Cd:Zn (0.52%) at day 30 displayed a high degree of ammonification followed by Cu: Cd which was 0.50%and the lowest value for Cd (0.19%) was found at day 60. Probably this is a result of small mineralization or rapid remineralization of nitrogen over a period of time. Ammonia volatilization and/or immobilization by the heterotrophic microorganisms could result in similar or lesser N to be mineralized in other treatments. The recalcitrant organic fractions might slow down N mineralization over time. Cd and Zn interaction may change the pH of soil [35]. Cd toxicity increased which affect N mineralization in soil [36]. Worked on cadmium and zinc uptake in wheat and found similar results. The existence of antagonistic relationship between Cd and Zn prevails throughout the incubation time. This phenomenon enhances the nitrification by which nitrogen may be lost from the system. As a result, values of total nitrogen obtained were minimized. Another researcher found an antagonistic relationship between zinc and cadmium [36]. On the contrary, it is believed that an additive action or synergistic effect between Cu and Zn could have been responsible for the enhanced toxicity of Cu:Zn in the soil sample and was reported by [37]. The combination of Cd:Zn:Cu was less effective on the total nitrogen change. Based on a study, the addition of heavy metals reduced N mineralization in soil [38]. This phenomenon results from a decrease in nitrifying bacteria activity. However, metal variables revealed positive interactions with parameters and N-related indices such as urease, N-acteyl-glucosaminidase, total N mineralization, ammonification and metabolic quotient [39].

Table 9: Effects of heavy metals on changes of available iron in soil (mg kg^{-1})

Sample	O	A	B	C	D	E	F	G
Treatment days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	2.08	0.37	0.36	2.59	2.44	2.50	2.56	2.46
15	2.02	0.30	0.18	2.02	0.95	1.33	6.37**	7.32**
30	1.10	1.49	1.77	1.06	1.03	0.96	5.46**	1.21
45	0.96	0.49	0.89	1.17	1.31	0.89	0.78	0.85
60	1.14	0.60	0.53	1.17	0.92	0.85	0.50	0.57

**Asterisks indicate significant difference from the control treatment (N=8, P≤0.01)

From the Table 9, we found that as compared with control treatment there was no significant relationship among the heavy metal treatments when they are applied singly, but in combination a significant (p≤0.01) relationship exist in Cu:Cd and Cd:Zn:Cu treatments. In case of Cu:Cd, maximum mineralization (6.37 $mgkg^{-1}$) was found at day 15 and day 30 the result was 5.46 $mgkg^{-1}$ due to the synergistic interaction between them but after that the value decreased drastically due to antagonistic interaction between them. Kabata-Pendias [36] found both antagonistic and synergistic relation between Cu and Cd.

Table 10: Effects of heavy metals on changes of total iron in soil (%)

Sample	O	A	B	C	D	E	F	G
Treatment days	Control	Cd	Zn	Cu	Cd:Zn	Zn:Cu	Cu:Cd	Cd:Zn:Cu
0	3.63	4.11	2.14	1.85	2.14	1.96	1.85	3.63
15	3.24	3.60	2.98	3.40	3.45	3.02	3.32	3.22
30	4.68	1.42**	1.17**	0.14**	1.95*	5.74	1.38**	1.60**
45	3.02	0.88*	0.79*	0.30	0.36**	0.36**	0.70*	1.61
60	2.36	0.53	0.84	0.15	0.19	0.16	0.32	1.95

*Asterisks indicate significant difference from the control treatment (N=8, P≤0.05); **Asterisks indicate significant difference from the control treatment (N=8, P≤0.01)

Initial mineralization took place in all the treatments until 15 days of the incubation together with the probability of Fe immobilization (Table 10). As compared to control, at day 30 except Zn:Cu, all treatments varied significantly (p≤0.05) over the incubation period. At day 45, all treatments showed the decreasing pattern and varied significantly except Cu and Cd:Zn:Cu. This may due to higher concentration of single metal along with the antagonistic interaction among the metals used helps the release of iron from exchange position. A scholar observed an antagonistic interaction between Cu and Zn. A scholar found an antagonism relation between Cd and Zn.Cd:Zn:Cu showed increment in total Fe at day 45 along with at the end of the incubation period [40]. This may due to the organism cell lyses. However, at the end of the incubation period no significant change in total Fe observed.

4. CONCLUSIONS

The effect of heavy metals applied in soil in single and in combination on the changes in carbon, nitrogen and iron mineralization revealed that the single metal treatment showed more effect on microbial activities than in combination. The combined effect showed interactions among the metals which was antagonistic in nature.

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