

Research Article

Contamination of Soil with Pb and Sb at a Lead-Acid Battery Dumpsite and Their Potential Early Uptake by *Phragmites australis*

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Recycling of spent Lead-Acid Batteries (LABs) and disposal of process slag potentially contaminate soil with Pb and Sb. Total and available concentrations of Pb and Sb in three soil treatments and parts of *Phragmites australis* were determined by atomic absorption spectrophotometry. Soil with nonrecycled slag (NR) had higher total metal concentrations than that with recycled slag (RS). Low available fractions of Pb and Sb were found in the soil treatments before planting *P. australis*. After 16 weeks of growth of *P. australis*, the available fractions of Pb had no statistical difference from initial values ($p > 0.05$) while available Sb fractions were significantly lower when compared with their initial values ($p < 0.05$). Metal transfer factors showed that *P. australis* poorly accumulate Pb and Sb in roots and very poorly translocate them to leaves after growing for 8 and 16 weeks. It may be a poor phytoextractor of Pb and Sb in metal-contaminated soil at least for the 16 weeks of its initial growth. However, the plant established itself on the metalliferous site where all vegetation had been destroyed. This could be useful for potential ecological restoration. The long-term phytoextraction potential of *P. australis* in such environments as LABs may need further investigation.

1. Introduction

Soil is a geochemical sink of contaminants and natural buffer for controlling the transport of elements to the atmosphere, hydrosphere, and biota [1]. The mobility and bioavailability of these elements in soil are controlled by geochemical, climatic, and biological factors [2]. Anthropogenic activities may render soil unsuitable for various land uses. Processes for recycling of Lead-Acid Batteries (LABs) and dumping of resultant slag may be important routes for soil contamination. Some trace elements emitted to the biosphere have toxic effects on the environment and human health (i.e., exposure to Pb and Sb) [3, 4]. People living near or working at LAB recycling sites and dumpsites may be exposed to Pb and Sb through contact with contaminated water, runoff, and airborne particulate emissions. Various physicochemical clean-up technologies relying on intensive soil manipulation [5] or bioremediation [6] have been used for the reclamation of contaminated soil. However, most of these technologies are

not cost-effective and environmentally friendly [1, 7]. Previous studies have focused on phytoremediation technologies [8–11] and available evidence suggests that the technologies are ecofriendly, innovative, and economical [12].

Human exposure to Pb and Sb may be occupational or nonoccupational. Lead has historically been used in water pipes, artifacts, LABs, and gasoline additive, tetraethyl-lead [13]. Antimony has been used as a fire retardant, in plastics, in coatings, in electronics, as a decoloring agent in glass, as alloys in LABs, and as a catalyst in the production of polyethylene terephthalate polymers [14]. The demand for energy and the subsequent widespread use of LABs by individuals, households, and industries result in large tonnage of spent batteries. Automobile LABs contain polypropylene, concentrated H_2SO_4 , Pb electrodes with either PbO_2 paste cathode or Pb anode, and various metals such as Sb, As, Cd, Sn, and Cu [15]. Spent LABs are hazardous materials [14] that need appropriate handling and disposal in specially designed facilities and not in conventional landfills. Recycling

of spent LABs may be more environmentally friendly than open dumping or incineration. According to Royer et al. [15], sites where LABs are recycled pose challenges for remediation due to a variety of soil contamination sources.

Phragmites australis (Cav.) Trin. ex Steud (common reed) is a widely distributed macrophyte throughout the world [16]. The terrestrial form of the plant has higher dry matter content and low growth rate and specific leaf surface area which makes it resist environmental stresses and adapt to adverse environments better than the aquatic form [17]. *Phragmites australis* can grow in environments of extreme pH and poor nutrient content [18]. Humans use the common reed to make woven mats, for fishing, as hunting traps, in musical instruments, or in baskets, among other applications. Animals browse the plant. *Phragmites australis* has reportedly been used for remediation of heavy-metal-contaminated aquatic ecosystems [19–21]. To the best of the authors' knowledge, limited information is available on the early uptake and accumulation of Pb and Sb by *P. australis* under field conditions, particularly in soil with recycled and nonrecycled slag from the recycling of automobile LABs.

The current study assessed the contamination of soil with LAB waste and the potential early uptake of Pb and Sb by *P. australis* under field conditions. The total and available concentrations of Pb and Sb in three soils (with recycled slag, nonrecycled slag, and reference) and total metal in three plant parts (root, stem, and leaf) of *P. australis* were determined. For remediation purposes, the study site required a plant species which could survive with limited water supply such as seasonal rainwater once established. We hypothesized that the common reed can adapt to such an environment and once introduced it could reestablish an ecosystem where other plants may grow. Then, it would take up Sb and Pb during its early stages of development. The identification of plant species for phytoremediation may help in ecological restoration of contaminated environments.

2. Materials and Methods

2.1. Description of the Study Site. An automobile LAB dumpsite located in Norton (17°52'11"S, 30°41'24"E), a small town 46 km west of Harare, Zimbabwe, was studied. Lead-Acid Battery waste was discharged directly onto a one-hectare dumpsite, formerly a marshy area. It appears that the phytotoxic effects of the contaminants were severe since all indigenous vegetation had been destroyed (Figure 1(b)). Operations that generate LAB waste include automobile battery breaking (Figure 1(a)), smelting, and refining. Field work conducted on the dumpsite entailed planting vetiver grass and *P. australis*, of which the former subsequently died within three days (Figure 1(b)) but the latter survived (Figure 1(d)). The study site had fersialitic or chromic luvisol or rhodic paleustalf soil [22].

2.2. Sampling and Chemical Analyses. The experimental design consisted of three treatments which were replicated three times: soil with nonrecycled slag (NR), recycled slag (RS), and a reference site (RF). The reference site was 5 km away in the windward side. Three similar beds measuring

15 m² (5 m × 3 m) were prepared for each treatment. Each bed consisted of three rows with inter- and intrarow planting of 1 m × 1 m. It was raised to 30 cm in order to avoid ground or near-neighbor effects [23]. About 0.50 m was left from the edges. Three of the 15 established planting points (20%) from each bed were randomly selected for sampling the growth media (0.5 kg) to a depth of 30 cm using a soil auger. This was repeated for another two treatments. Split samples were used for the determination of selected physicochemical parameters. Remaining samples were oven-dried at 105°C for 1 h and then at 80°C for 24 h (Heraeus D6450) [24], ground, and sieved (<1 mm). These were used for the determination of other parameters including the total and available metal concentrations. The pH was determined using a glass pH meter (micropH 2002) in a 1:1 soil/water suspension. Electrical conductivity (EC) was measured using a conductivity meter (ERMA EC035). The NO₃-N concentration of soil was determined by extraction with 2 M KCl (1:10, m/v) and analyzed by colorimetry (UV-Vis spectrometer model GENESYS 10S, Thermo Scientific, Germany) [25]. Available phosphorus was determined as PO₄-P by extraction from soil with 0.5 M NaHCO₃ (42 g in 1 L) at pH 8.5 and then measured colorimetrically (UV-Vis spectrometer model GENESYS 10S, Thermo Scientific, Germany) using acidified blue ammonium molybdate [26]. The Walkley-Black method was used for determining organic matter [27]. In this procedure, carbon is oxidized by acidified dichromate and excess dichromate is back-titrated with ferrous iron with diphenylamine indicator. Soil textural composition was determined using the Bouyoucos hydrometer method [28]. Total Pb and Sb concentrations in the filtered digests were determined spectrometrically (Flame AAS; GBC-Savant).

Two split powdered growth media samples from each treatment (1 g and 0.5 g) were separately used for total (*aqua regia*) and available (ammonium acetate) Pb and Sb extraction. A 1 g sample was digested in an acid mixture (HCl and HNO₃, 20 ml, 1:3, v/v) on a hot plate (110°C, 3 h) until decomposition was complete. This was then evaporated to reduce volume to about 5 ml. Digests were filtered (Whatman filter paper No. 1), washed with deionized water, and transferred quantitatively to a 50 ml volumetric flask. Neutral ammonium acetate (1 M CH₃CO₂NH₄, pH 7) (10 ml) was added to the 0.5 g split sample of powdered growth media sample in a 250 ml beaker. The sample was shaken in a multishaker (Kahn Shaker, 140 rpm, 1 h) and filtered (Whatman filter paper No. 1) into a 50 ml volumetric flask which was completed to the mark with 1 M CH₃CO₂NH₄. This was replicated three times for each treatment. The concentrations of Pb and Sb in sample solutions were determined spectrophotometrically (Flame AAS; GBC-Savant) using appropriately prepared specific calibration curves. After 16 weeks of growth of *P. australis*, growth media in the rooting zone were sampled and analyzed for both total and available Pb and Sb. A reagent blank was run ten times. Metal recovery studies for growth media were carried out using a certified reference material (CRM) (channel sediment BCR 320R: 0083) [29].

Rhizomes (96) of *P. australis* (30–53 cm long) with some roots were taken from adult plants at a presumably unpolluted (with Sb and Pb) site. These were cut to approximately



FIGURE 1: (a) Battery breaking and (b) slag dumpsite with dead vetiver grass within 3 days of planting, at a Lead-Acid Battery recycling and smelting site in Norton, Zimbabwe. (c) *P. australis* rhizomes before preparation for planting and (d) *P. australis* growing in an experimental bed of soil with recycled slag.

25 cm each to give 157 rhizomes. Ten randomly selected cut rhizomes (6.4%) were repeatedly washed with stream water and then with deionized water. They were oven-dried (Heraeus D6450; 70°C, 24 h), ground, and sieved to <1 mm [12]. Ten 1.0 g split dried samples were separately decomposed in a muffle furnace (550°C, 6 h) and the ashes were dissolved in *aqua regia* (12 ml). These were filtered (Whatman filter paper No. 1) into 25 ml volumetric flasks which were completed to the mark with double-distilled water and analyzed for Pb and Sb using FAAS (GBC-Savant) [21]. The remaining cut rhizomes (145) were planted in nine beds (three beds for each treatment) at a depth of about 20 cm and watered with 40 L of tap water (20 L polyethylene bucket) per bed skipping a day or two in between watering events. No rainfall events were recorded during the study period. After eight weeks of planting, three whole plants of *P. australis* were randomly harvested from each bed within and across treatments. Composite plant part samples (leaves, stems, and roots) were made for each treatment. A garden hoe was used to uproot plants while a pair of secateurs was used to cut leaves. Plant tissues were separately put in open polypropylene bags, labeled, and sent to the laboratory. Each sample was washed with a jet of tap water and then with

distilled water. Oven-dried samples were ground to a fine powder and thoroughly mixed. Laboratory samples were then prepared as described above for the growth media in order to determine Pb and Sb concentrations. Bioaccumulation and translocation factors were calculated [12]. Lead and Sb were extracted from two sets of three replicate samples of the CRM by acid digestions and the other set was extracted by ammonium acetate and analyzed using similar procedures as the growth media. A procedural blank was run ten times for Pb and Sb analysis.

2.3. Quality Control and Statistical Procedures. During sampling, plant parts affected by herbivory or with signs of disease were avoided. Composite samples were used and replicated three times. Reagents blanks and calibration standards were run in between sample analyses. All apparatuses used were washed thoroughly before use and then rinsed thrice using deionized water. Analytical-grade reagents were used in all the analyses. A certified reference material was used to validate the analytical procedure. IBM SPSS statistical package version 21 was used for data analysis. Significant differences among physicochemical parameters for the three treatments were determined using One-Way ANOVA and

LSD post hoc test. All tests were considered significant at $p < 0.05$. A paired sample t -test was used to compare the concentrations of Pb and Sb in the growth media before planting and 16 weeks after planting.

3. Results and Discussion

3.1. Physicochemical Properties of Growth Media. Element recovery studies of a CRM gave 95.8% (81.43 ± 1.73 mg/kg) and satisfactory RSD (2.12%) for total Pb. Limits of detection (LODs) were 0.001 mg/L (0.10 mg/kg) for Pb and 0.003 mg/L (0.3 mg/kg) for Sb. Mean concentrations of Pb and Sb in 10 rhizomes before planting for the three treatments were less than the LOD. Table 1 shows the physicochemical parameters of the growth media before and after 16 weeks of planting *P. australis* in three soil treatments (NR, RS, and RF). Soil with recycled slag (RS) generally had significantly higher clay content and pH but lower organic matter (OM) and nutrients (NO_3^- , PO_4^{3-}) than soil with nonrecycled slag (NR) before and after 16 weeks of planting *P. australis* ($p < 0.05$). The concentrations of Pb and Sb in the soil treatments decreased in the order NRS > RS > RF. After 16 weeks of planting, the concentrations of NO_3^- (NRS and RS) and OM (RF) were significantly lower than their initial values before planting (t -test, $p < 0.05$). Not significantly different initial and final soil parameters after 16 weeks could be explained by the absence of rainfall events during the study period (except for occasional initial watering) which could promote leaching, overall insignificant element uptake by *P. australis* (for Sb), and additive effects of atmospheric deposition from the adjacent LAB recycling processes. However, leaching of Sb and Pb could not be excluded through watering but to a lesser extent as the two elements are strongly bound by soil clay minerals and organic matter [1]. High pH of slag soil treatments (10.7–12.1) may not favor mineralization of Pb and Sb. The observed significant decrease in soil Sb after 16 weeks in NR could be attributed to plant uptake, leaching, and other soil biochemical processes. There were no significant differences in the concentrations of Pb before planting and 16 weeks after planting of *P. australis* for both NRS and RS treatments ($p > 0.05$).

US EPA [5] reported 80,000 mg/kg Pb at LAB recycling sites. Soil that was 60 m away from an abandoned battery waste site had 41,890 mg/kg Pb [30]. At another abandoned scrap deposit site, soil had 104,000 mg/kg Pb [24]. This shows that LAB wastes are a very important route for the release of trace elements into the environment. People living near or working at sources of Sb and Pb such as smelters, coal fired plants, and refuse incinerators may be exposed to toxic elements in dust, soil, and vegetation. A study of the exposure of residents and children living in a battery recycling craft village in Vietnam showed Pb-contaminated hair, blood, and urine [31]. Similar observations of increased blood Pb levels were made in another study at a LAB recycling and manufacturing plant in Kenya [32]. Recycling processes may not be very efficient in recovering metals. Slag from recycling LABs still contains up to 5% Pb [13, 14]. Other than direct disposal of slag on the dumpsite, toxic elements may be added to the soil surface by atmospheric deposition of fugitive dust

and drainage or leachate from waste heaps. Soil naturally contains trace Sb at concentrations of less than 1 mg/kg (average: 0.48 mg/kg) but values of 109–2,550 mg/kg Sb from processing sites were reported [3]. An average background concentration of 0.67 mg/kg Sb was also reported [1].

Clay mineral content, organic matter, phosphorus, and moisture content of a soil are very important parameters that influence the bioavailability and uptake of trace elements by plants [1, 2, 33]. Results from the current study clearly show that soil at the LAB dumpsite was contaminated with Sb and Pb. Exposed populations may be encouraged to have Pb levels in their blood, hair, and urine monitored for possible adverse health effects.

3.2. Concentrations of Sb and Pb in Plant Parts of *P. australis*. Table 2 shows the concentrations of Sb and Pb in plant parts of *P. australis* grown in three different soil treatments (NR, RS, and RF) after 8 and 16 weeks of planting. The recycled slag treatment (RS) had lower concentrations of Pb and Sb for roots than the nonrecycled slag soil treatment (NR) ($p < 0.05$). No significant differences were observed for the concentrations of both elements between NR and RS treatments for leaves and stems after 8 and 16 weeks of planting *P. australis* ($p > 0.05$). A paired t -test to compare elemental concentrations within a soil treatment between 8- and 16-week growth periods showed significant differences ($p < 0.05$) for Pb and Sb in roots (NR and RS) only. Both metals were not detected in all plant parts from the RF treatment. Concentrations of Pb and Sb decreased in the order root > leaf > stem for NR and RS treatments. Results suggest that Pb and Sb were coming from LAB slag.

Table 3 shows that the Biological Absorption Factor (BAF) and Translocation Factor (TF) were less than unity for element transfer. These indicate that, after 8 and 16 weeks of planting, *P. australis* poorly accumulate Pb and Sb into roots and poorly translocate them to leaves. Plant roots appeared to take up more Sb than Pb in both NR and RS soil treatments after harvesting at 16 weeks. *Phragmites australis* appeared to take up more Pb after 16 weeks (3.3-fold) and Sb (5.5-fold) in the NR soil treatment and more Pb (5-fold) and Sb (4.5-fold) in the RS soil treatment than it did after 8 weeks of growth.

The above-ground : below-ground concentration ratios of Sb and Pb were very small (less than 0.15) in NR and RS soil treatments. This may suggest that the common reed (*P. australis*) is poor for phytoextraction of Pb and Sb from contaminated soil. A translocation factor well above one indicates a possible candidate for phytoextraction [10]. In its various remediation applications in contaminated aquatic ecosystems, *P. australis* showed that it is a poor phytoextractor of Pb and Sb but good for phytorhizofiltration [19, 34, 35]. One drawback in this field experimental setup, and thus phytoremediation of trace elements from contaminated soil, is the failure to control leaching of trace elements. Particulate fallout and foliar absorption of Pb cannot be excluded in this study since the element has been reported to be poorly translocated to leaves [1], yet Pb and Sb were recorded in appreciable amounts in the current study. Establishment of vegetation in an area where all native plants were destroyed was fascinating. This development may help filter and reduce

TABLE 1: Physicochemical characteristics of three soil treatments replicated three times (nonrecycled slag, recycled slag, and reference) at a LAB dumpsite in Norton, Zimbabwe. Parameters were determined before planting and 16 weeks after planting of *P. australis*. Values are expressed as mean \pm SD of triplicate measurements.

Soil parameter	Before planting			16 weeks after planting			Reference soil
	Nonrecycled slag treatment	Recycled slag treatment	Reference soil	Nonrecycled slag treatment	Recycled slag treatment	Reference soil	
Clay%	6.33 \pm 1.53	12.33 \pm 1.53	18.67 \pm 2.52	7.67 \pm 1.63	10.63 \pm 1.64	21.60 \pm 2.95	
Silt%	54.00 \pm 2.65	61.00 \pm 4.00	34.00 \pm 3.00	55.10 \pm 3.92	59.77 \pm 1.53	34.60 \pm 0.40	
Fine sand%	39.67 \pm 1.53	26.67 \pm 2.52	47.33 \pm 0.58	37.23 \pm 4.52	29.60 \pm 2.91	43.80 \pm 3.17	
Moisture content (g)	4.53 \pm 0.22	6.76 \pm 0.04	12.80 \pm 0.30	—	—	—	
pH (H ₂ O)	10.90 \pm 0.36	12.10 \pm 0.20*	7.23 \pm 0.06	10.70 \pm 0.10	10.93 \pm 0.15*	7.17 \pm 0.06	
PO ₄ ³⁻ (mg/kg)	0.85 \pm 0.12	0.50 \pm 0.02*	1.45 \pm 0.03	0.74 \pm 0.09	0.58 \pm 0.03*	1.37 \pm 0.17	
NO ₃ ⁻ (mg/kg)	0.61 \pm 0.04*	ND	1.37 \pm 0.05	0.55 \pm 0.07*	ND	1.30 \pm 0.08	
EC (μ S/cm)	144.50 \pm 12.08	85.80 \pm 8.35	41.03 \pm 4.44	152.17 \pm 8.92	108.40 \pm 16.59	38.67 \pm 1.82	
Organic matter (%)	0.93 \pm 0.06	0.31 \pm 0.03	3.11 \pm 0.18*	0.87 \pm 0.09	0.38 \pm 0.03	3.57 \pm 0.05*	
Total Pb (mg/kg)	48,840 \pm 4,000	27,103 \pm 3,869	0.51 \pm 0.05	43860 \pm 7,066	23,380 \pm 2,495	ND	
Total Sb (mg/kg)	3,460 \pm 645*	2,583 \pm 523	ND	2,343 \pm 531*	2,163 \pm 172	ND	
Available Pb (mg/kg)	278.41 \pm 20.50	111.54 \pm 5.81	—	230.08 \pm 42.33	95.34 \pm 34.16	—	
Available Sb (mg/kg)	86.31 \pm 13.08*	64.83 \pm 8.32*	—	49.68 \pm 16.36*	42.54 \pm 8.24*	—	

ND: not detected (below LOD). — (dash): parameter not determined. All parameters were significantly different across the three treatments before planting ($p < 0.05$). *Parameters significantly different before planting and after planting of *P. australis* for a given soil treatment (paired *t*-test; $p < 0.05$).

TABLE 2: Concentrations of Pb and Sb in tissues of *P. australis* harvested from different soil treatments after 8 and 16 weeks of planting. Values are expressed as mean \pm SD of triplicate measurements in mg/kg, DW.

Element	Growth period (wks)	Nonrecycled slag treatment			Recycled slag treatment			Reference soil treatment		
		Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem
Pb	8	136.57 \pm 18.91 ^{a*}	8.77 \pm 0.90 ^a	2.88 \pm 0.27 ^a	98.90 \pm 12.76 ^{b*}	7.83 \pm 1.74 ^a	2.63 \pm 0.30 ^a	ND	ND	ND
	16	590 \pm 36 ^{a*}	8.61 \pm 1.04 ^a	2.76 \pm 0.38 ^a	466.67 \pm 25.17 ^{b*}	8.04 \pm 0.23 ^a	2.96 \pm 0.12 ^a	ND	ND	ND
Sb	8	67.83 \pm 9.97 ^{a*}	3.33 \pm 0.36 ^a	1.45 \pm 1.91 ^a	47.84 \pm 3.86 ^{b*}	2.89 \pm 0.19 ^a	1.56 \pm 0.23 ^a	ND	ND	ND
	16	260 \pm 20 ^{a*}	3.47 \pm 0.32 ^a	1.73 \pm 0.171 ^a	205 \pm 18 ^{b*}	2.94 \pm 0.21 ^a	1.50 \pm 0.16 ^a	ND	ND	ND

ND: not detected (<LOD). Different superscripts (a, b) in a row denote significantly different concentrations ($p < 0.05$) of a given element for a plant tissue across treatments; * in a column denotes significantly different concentrations (paired t -test, $p < 0.05$) for a given element for a specific plant tissue between 8- and 16-week growth periods within a soil treatment.

TABLE 3: Bioaccumulation and translocation factors of Pb and Sb for *P. australis* in different soil treatments after 8 and 16 weeks of planting.

Element	Growth period (wks)	Nonrecycled slag treatment		Recycled slag treatment		Reference soil treatment	
		$[E]_r/[E]_s$	$[E]_l/[E]_r$	$([E]_l + [E]_{st})/[E]_r$	$[E]_r/[E]_s$	$[E]_l/[E]_r$	$([E]_l + [E]_{st})/[E]_r$
Pb	8	0.003	0.06	0.09	0.004	0.08	0.11
	16	0.01	0.01	0.02	0.02	0.02	0.02
Sb	8	0.02	0.05	0.07	0.02	0.06	0.09
	16	0.11	0.01	0.02	0.09	0.01	0.02

[E]_r: concentration of the element in roots of plant; [E]_s: concentration of the element in soil on which the plant is growing; [E]_l: concentration of the element in leaves of plant; [E]_{st}: concentration of the element in the stem of plant.

surface runoff laden with contaminants. It will be interesting to find out the nature of other plants that would be established on the site with time and how *P. australis* may take up Pb and Sb.

4. Conclusions

Contamination of soil with LAB recycling wastes and potential early uptake of Pb and Sb by *P. australis* after 8 and 16 weeks of planting were studied at a dumpsite. Slag and LAB recycling processes introduced large quantities of Pb and Sb into soil, altered soil characteristics, and destroyed indigenous vegetation. Soil with recycled slag had lower nutrient (NO_3^- , PO_4^{3-}) content, OM, and trace elements (Pb, Sb) than soil with nonrecycled slag. Uptake of Pb and Sb by roots of *P. australis* appeared to increase over time although the poor accumulation in leaves and stems appeared to remain constant. The NR soil treatment appeared to promote root uptake of Sb compared to Pb. *Phragmites australis* poorly accumulates Pb and Sb into roots and poorly translocates them to leaves, and thus it is a poor candidate species for phytoextraction in contaminated field soil conditions. However, its ability to be established on a site where all vegetation had been destroyed could further be explored as a starting point for ecological restoration. Accumulation of Pb and Sb by *P. australis* in the long term and possibilities of whether other plant species would get established on this site may need further studies. Based on findings and results obtained in the current study, the authors recommend biological monitoring of Pb in urine, hair, and blood samples for LAB recycling workers and populations residing near LAB smelters and dumpsites.

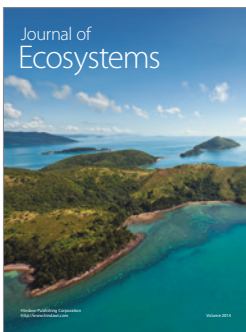
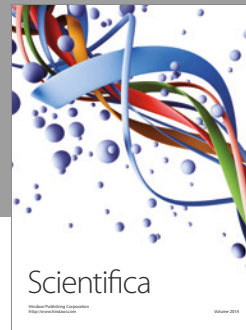
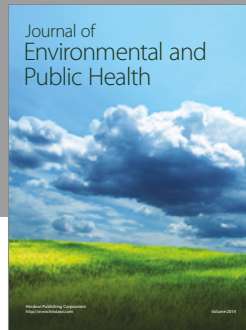
Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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