

Sulphide mining as a source of arsenic in the environment

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Based on the analysis of various types of environmental samples in the mining and smelter areas associated with the Khetri Cu and Zawar Pb-Zn deposits, it is concluded that zones of exploitation of all sulphide-bearing mineral deposits are potential areas of arsenic (As) contamination. Without identifying the source of As and various natural and anthropogenic concentrating mechanisms for As, such as natural scavenging of As by Fe-Mn hydroxides in sediments and industrial concentration of Cu, Pb and Zn along with the associated As in smelter operations, the pollution and health problems, as known in West Bengal and Bangladesh, cannot be suitably tackled.

THE presence of arsenic in groundwater poses a threat to human health due to its toxic properties¹. The World Health Organisation (WHO)² and the US Environmental Protection Agency (US-EPA)³ have set 10 ppb as the limit for arsenic (As) in drinking water. The problems of arsenic contamination of groundwater in West Bengal (India)⁴⁻⁷ and Bangladesh⁸⁻¹⁰ have been widely reported. In its geological occurrence, arsenic is found at high levels in primary sulfide minerals such as arsenopyrite (FeAsS), pyrite (FeS), pyrrhotite (Fe_{1-x}S), orpiment (As₂S₃) and realgar (AsS)¹¹. The estimated large arsenic deposits throughout the world are about 8.65×10^6 metric tonnes (t)¹². In terms of origin, the hydrothermal volcanic/sedimentary arsenic constitutes 4.5×10^6 (t), followed by hydro-mesothermal arsenic 3.61×10^6 (t) and hydro-epithermal arsenic deposits 0.544×10^6 (t)¹². The major period of arsenic deposits occurred in the world during carboniferous (about 65% of all As deposits) while at other geological times they are scattered in small quantities¹². In addition to substitution in sulphide deposits, its ionic radius and valence allow arsenic to substitute in trace amounts for many elements in silicates such as glauconite and other rock-forming silicate minerals^{11,13}. The chemical similarities of arsenate (H₂AsO₄⁻) and phosphate (H₂PO₄⁻) lead to anion solid solutions between minerals such as pyromorphite Pb₅(PO₄)₃Cl and mimetite Pb₅(AsO₄)₃Cl (refs 11 and 14). Combining both primary minerals and trace substitution in other minerals, arsenic has an estimated average concentration of 1.5 µg/g in the upper crust^{11,15}.

In surficial water systems, arsenic can be dissolved either directly from weathering of minerals^{11,16} or through mixing with high-arsenic bearing geothermal

waters^{11,17}. In groundwater systems, arsenic can also be derived from the dissolution of arsenic-bearing iron oxides in unconsolidated aquifers^{11,18}. Aqueous arsenic concentrations are controlled by anion exchange and coprecipitation with iron and manganese oxyhydroxides, and are therefore a function of Eh and pH¹¹. Arsenate anion exchange dynamics are analogous to phosphate, with competition for exchange sites favouring phosphate over arsenate¹¹. Competitive exchange reactions show that arsenic can be stored in soils, and then inadvertently released with the addition of a high phosphate source, such as phosphate fertilizer^{11,19}.

Thus, As is a highly mobile element in our environment and sulphide mineral deposits even if they contain only traces of As are potential contributors of this toxic element to the environment. It was reported²⁰ that the sulphosalts of Rampura-Agucha contain enhanced As levels but no such reports are available for the major sulphide mineral belt. Accordingly, an attempt was made to understand the source of As, its movements in the mine waters and its enrichment in the sulphide mining zones of Khetri and Zawar mines and concentrates in smelter operations.

Surface water samples were obtained from Zawar (Pb and Zn sulphides) and Khetri (Cu sulphide) mines and also groundwater from the surrounding residential areas were collected. Figure 1 shows the sampling sites and other mineral mining areas. It should be noted that some of the other mining areas have similar minerals and are therefore potential sites of arsenic contamination. Fresh grains of the sulphide ores, Pb, Zn and Cu concentrates and their tailings were also collected from the ore beneficiation plants at Zawar and Khetri. After collection, water samples were filtered through 0.45 µm membranes, acidified to pH 2 with HCl, and stored at about 4°C until further analysis. Ore, concentrates and tailings were digested in a Teflon bomb. Various geo standards (GXR2, GXR4, GXR5, GXR6, JB-1b, JB2, JCFA-1, JDO1, JG2, JLK1, JR2, JSD2, JSD3 and JSI1) were used for instrument calibration. Finally total arsenic in water and other samples were measured by an atomic absorption spectrophotometer (GBC 902) coupled with hydride generator (HG900) using hot vapour technique²¹. For water samples, chemical standards of As were used for calibration.

Table 1 shows the arsenic concentration in various water bodies in the mining areas. In groundwater (Zawar area) the total dissolved arsenic was between 1.4 and 13.8 ppb with an average of 5.8 ± 5.5 ppb. In the Khetri area the concentration was generally below <1 ppb (detection limit). The average total dissolved arsenic in Zawar and Khetri mine inlet water was 3.0 ± 2.8 ppb and <1 ppb, respectively while the mine outlet water had 130 ± 14 ppb and 3 ± 1 ppb arsenic, respectively. Oxidation of metallic sulphides under the action of inlet water produces sulphuric acid²² and the metals released

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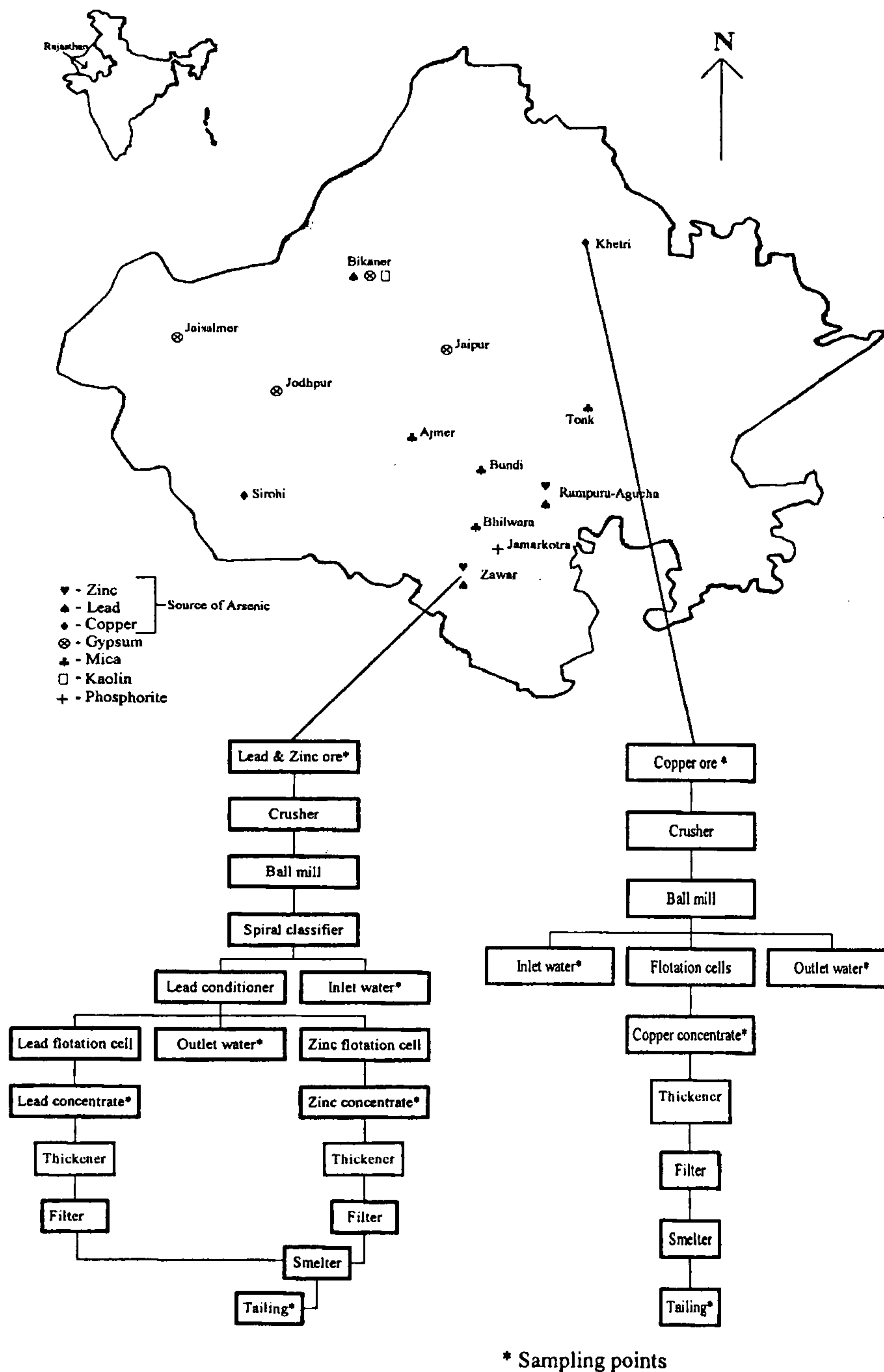


Figure 1. Map of Rajasthan showing sampling sites and flow sheet of ore beneficiation.

are soluble in this acidic water thus releasing arsenic to the outlet water. Arsenic concentration in lakes, rivers and groundwater in other parts of the world is shown in Table 2 for comparison. Our analysis shows higher concentration of arsenic in Zawar and Khetri ore with an average of 7683 ± 4414 mg As/kg and 145 ± 72 mg As/kg of the sulphide ores, respectively. Table 3 shows the average concentration of Zawar and Khetri ore and tailings with reference to Rampura-Agucha sulphosalts and compares them to other world sulphide ores and tailings. In the second part of Table 3, arsenic enrich-

ment in various aquatic environments such as rivers and lakes are indicated. The average concentration of arsenic in Zawar zinc and Khetri copper tailings was 1519 ± 1493 mg As/kg and 1179 ± 1382 mg As/kg of tailings, respectively. In the metal sulphide smelter, in the zinc concentrate 10727 ± 6322 mg As/kg of the concentrate, in the Pb concentrate 61 ± 16 mg As/kg of the concentrate and in the copper concentrates 12 ± 6 mg As/kg of the concentrate were observed. Processing of Pb-Zn sulphide ores involves first the separation of Zn from the Pb concentrates through addition of chemicals

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Table 1. Total arsenic in groundwater (ppb), mine water (ppb) and in the sulphide ore, smelter concentrates and tailings (mg/kg) in the Khetri and Zawar region

Zawar	GW	MW ¹	MW ²	As in ore	As in Zn conc. ^x	As in Pb conc. ^x	As in tailings
1	4.7	6.9	120	3262	6256	49	463
2	13.8	0.9	140	8410	15197	72	2575
3	1.4	0.9	—	5558	—	—	—
4	3.3	3.3	—	13501	—	—	—
Mean	5.8	3.0	130	7683	10727	61	1519
SD	5.5	2.8	14	4414	6322	16	1493
Khetri	GW	MW ¹	MW ²	As in ore	As in Cu conc. ^x		As in tailings
1	tr	tr	3	70	7		202
2	tr	tr	2	202	16		2156
3	tr	tr	—	105	—		—
4	tr	tr	—	180	—		—
5	tr	tr	—	74	—		—
6	tr	tr	—	241	—		—
Mean	tr	tr	3	145	12		1179
SD	tr	tr	1	72	6		1382

GW, groundwater; MW¹, mine water inlet; MW², mine water outlet; x, concentrates in the smelter; tr, trace (<1 ppb).

Table 2. Dissolved/particulate arsenic levels in diverse environments

Place	Nature of water	As (ppb)
Baitrani, India ³⁵	River	0.1–2.1
Bangladesh ⁸	GW	3–960
Bowen Island, British Columbia ³⁶	Well	0.5–580
Brahmani, India ³⁵	River	0.9–13.2
Bunker hill mine, Idaho ³⁷	GW in level (5 and 9)	0.1–1336
Caron River, England ³⁸	Water	1–80
Caron River, England ³⁸	Particulate	1–3800
Coeur d'Alene, Idaho ³⁷	River aquifer	0.17–2.87
East coast of India ³⁹	Pulicat lake	35
Fairbanks, Alaska ⁴⁰	Stream water	<5–1260
Furtei, South Sardinia ⁴¹	Borehole water	382
Hamilton, New Zealand ²⁵	Waikato river	32.1
Humber Estuary, NE England ⁴²	River	0.54–4.07
Madison river vally, Montana ⁴³	Alluvial GW	26–150
Mahanadi, India ³⁵	River	0.1–3
Missouri, USA ²³	Well	30–400 As(III)
New Hampshire ¹²	Drinking water	<0.0003–180
Northwest Arizona ⁴⁴	GW	10–1000
Ohakuri, New Zealand ²⁶	Lake	38.3–441
Quebec and Ontario, Canada ²⁴	Lake	97–7297
South Australia ⁴⁵	Coastal water	1.1–1.61
South Australia ⁴⁵	Particulate	<0.00006–0.013
South McQuesten, Canada ⁴⁶	River	<40–220
West Bengal, India ⁴	GW	10–3700
Khetri and Zawar, India ^P	GW	<1–13.8
Khetri and Zawar, India ^P	MW ¹	<1–6.9
Khetri and Zawar, India ^P	MW ²	2–140

GW, groundwater; P, present study; MW¹, mine water inlet; MW², mine water outlet.

such as potassium ethyl xanthate leading to enhancement of As in the Zn concentrates relative to the Pb concentrates. On the other hand, in the beneficiation of Cu ore, while the concentration of As in the Cu ore is less relative to that in Pb-Zn ores, even the small excess As is eliminated in liquid form in the tailings. Thus, the Cu beneficiation is more harmful to the environment due to

direct mixing of As enriched tailing with the immediate aquatic systems compared to the Zn beneficiation where As is enriched in the Zn concentrates and hence is in solid form.

From Table 3 it can be seen that As is introduced into the aquatic environment through sulphide mining activities. Lithologic logs in North America²³ show As asso-

Table 3. Dispersal of arsenic from source to aquatic environment

Place	Nature of sample	Primary minerals	Valuable elements	As (mg/kg)	Cu %	Fe %	Pb %	Zn %	S %
Khetri, Jhunjhunu ^P	Ore	ccp	Cu, Co	145	4.3	59.6	-	0.05	35.8
Khetri, Jhunjhunu ^P	Unoxidized tailings	-	-	1179	0.09	-	-	0.07	-
Zawar, Udiapur ^P	Ore	sp	Ag, Pb and Zn	7683	0.03	6.7	3.7	21.5	34.2
Zawar, Udiapur ^P	Unoxidized tailings	-	-	1519	0.01	-	-	0.15	-
Rampura-Agucha, Bhilwara ²⁰	Sulphosalt Freibergite	sp	Pb and Zn	9300	15.06	5.46	-	0.8	21.79
Rampura-Agucha, Bhilwara ²⁰	Sulphosalt Argentinian pearcite	-	-	5000	5.13	0.01	0.15	-	15.43
Bowen Island,	Vein deposits	asp	-	16.4-17.2	-	7.0-7.3	0.005-0.01	0.01	8.0-9.0
British Columbia ³⁶	Playa brine	-	B, Li	100	-	-	-	-	-
California, USA ¹¹	Ore	ccp, sp, gn	Au and Ag, Zn	98	1.3	21.2	0.04	14.1	24
Cleveland Deposits,	Tailings	-	-	4-190	0.07-3.1	2.1-36.8	0.01-0.2	0.02-2.2	4.5-17.1
New Mexico ⁴⁷	Ore concentrate	ccp, sp, gn	Cu, Fe with Au & Ag	290	10	24	0.1	0.9	24.4
Cyprus-Pinos Altos,	Tailings	-	-	42-110	0.07-0.9	10-15	0.01-0.1	0.01-0.1	1-3
New Mexico ⁴⁷	Cobaltite mineral	col	Co	44.8-47.4	-	2.0-4.08	-	-	19.5-20.5
Ingaldhal, Chitradurga,	Ore	ccp, sp, py	-	1200	1.03	34.15	0.4	10.61	42.14
Karnataka ⁴⁸	Unoxidized tailings	ccp, po	Cu	68	0.1	2.4	0.003	0.1	0.8
Killingdal, Norway ⁴⁹	Oxidized tailings	-	-	109	0.03	2.7	0.004	0.02	0.3
Laver, Norrbotten, Sweden ⁵⁰	Volcanic marine sediments	-	-	1200-4900	0.01	17.7-18.4	0.001	0.003	52.8-53.6
Laver, Norrbotten, Sweden ⁵⁰	Ore	ccp, sp, py	Cu, Zn	40	2.2	37.5	0.02	1.9	41.4
Li Hir Island, Papua New Guinea ⁵¹	Slag tailings	-	Ag, Au, Cu, Pb and Zn	3800	0.4	-	0.07	2.86	-
Lokken, Norway ⁴⁹	Tuff	-	Ag, Cu, Pb, Zn	45	0.003	1.8	0.004	0.01	0.88
Maria De La Paz, Mexico ⁵²	Rhyolite	-	Ag, Cu, Pb, Zn	62	0.001	1.3	0.001	0.02	0.3
New Brunswick, Canada ⁵³	Empire zinc mine tailings	ccp, sp	Ag, Au, Cu, Fe, Pb and Zn	3.8-185	0.05	-	0.1	3.8	-
New Brunswick, Canada ⁵³	Ore field	-	Cu, As, Se	6000	-	-	-	-	-
New Mexico, USA ⁵⁴	Ore	ccp, sp, py	-	500	1.3	-	-	1.7	45
Rio Tinto, Spain ¹¹	Cupin mine, Contwoyto lake	-	Au, Ag, Cu	4.20	0.03	-	0.001	0.003	20
Skorovas, Norway ⁴⁹	Volcanic hosted	stn	Ag, Au, Sb	638-7032	-	-	-	-	-
Slave Province, N. America ⁵⁵	Sediment or volcanic hosted	stn	Ag, Au, Sb	12-3109	-	-	-	-	-
South America ⁵⁶	Sediment hosted	stn	Ag, Au, Sb	1-326	-	-	-	-	-
South America ⁵⁶	Gabellino (floatation tailings)	ccp, gn, po, py, sp	Cu	50-388	0.03	-	0.2	0.6	-
South America ⁵⁶	Fontalcinaldo (waste rock dump)	-	-	55-1075	0.003	-	0.03	0.003	-
Southern Tuscany, Italy ⁵⁷	Unoxidized tailings	py, po, sp, ccp	Cu and Zn	1178	0.2	9.7	0.159	0.66	17.5
Southern Tuscany, Italy ⁵⁷	Ore	-	Ni, Cu	2.2-26	-	-	-	-	-
Stekeljokk, Sweden ⁵⁰	Arsenian pyrite	-	Au (595-1465 ppm)	1.05-2.43	-	-	-	-	-
Sudbury, Ontario ²⁷	Nevoria mine	-	Au, Ag, Cu	500	0.023	-	0.001	0.003	10
Twin Creeks, Nevada ⁵⁸	Marvel Loch mine	-	Au, Ag, Zn	2875	0.008	-	0.01	0.02	2.5
Western Australia ⁵⁵									
Western Australia ⁵⁵									

Table 3. (Contd.)

Bangladesh ^{59,60}	Ganges (Padma) river sediments	-	-	1.18-2.61	0.002-0.003	2.5-10.6	0.001-	0.005-	-
Bangladesh ^{59,60}	Brahmaputra (Jamuna) river sediment	-	-	1.41-5.93	0.002-0.005	3.1-6.0	0.005	0.01	-
Bangladesh ^{59,60}	Meghna river sediment	-	-	1.3-5.55	0.001-0.007	3.8-9.0	0.004	0.02	-
Blackbird creek, Idaho ⁶¹	Stream sediments	col, ccp, py	Co	42-2550	0.1-0.3	-	0.8-12.9	-	-
Bullit County, Kentucky ⁶²	New Albany raw shale	oil shale	-	14	0.03	2	0.003	0.001	1
Central Newfoundland, Canada ⁶³	Lower till (pyrite 5%)	asp, gn, sp, py	-	10-20	-	2.3	0.02-0.1	0.04-0.2	2.7
Central Newfoundland, Canada ⁶³	Meltout till (pyrite 10-20%)	-	-	30-90	-	4.7-5.3	0.08-0.02	0.2-0.8	7.0-8.0
Elba-Argetario, S. Tuscany, Italy ⁶⁴	Sediments	-	-	4-120	0.01	-	0.01	0.02	-
Fairbanks, Alaska ⁴⁰	Suspended sediments	au, stn, gn, sel	Au	0.005-18	-	-	-	-	-
Fairbanks, Alaska ⁴⁰	Sediment	-	-	5-4114	-	-	-	-	-
Fal Estuary, Cornwall, UK ⁶⁵	Tresillian river sediment	asp, ba, ccp, py, sp	-	508	0.1	-	0.05	0.2	-
Indian subcontinent ⁶⁶	Ganges river sediment	-	-	2.0-9.0	0.002	2.51	0.003	0.006	-
Indian subcontinent ⁶⁶	Brahmaputra river sediment	-	-	2.0-6.0	0.005	2.9	0.001	0.005	-
Indian subcontinent ⁶⁶	Yamuna river sediment	-	-	3.0-11.0	0.006	2.43	0.003	0.006	-
Indian subcontinent ⁶⁶	Narmada and Tapi river sediment	-	-	3.0-5.0	0.001-0.005	1.09-3.14	0.001	0.002-	-
Indian subcontinent ⁶⁶	Godavari river sediment	-	-	4.0-14.0	0.005	5.85	0.001	0.005	-
Indian subcontinent ⁶⁶	Krishna river sediment	-	-	2.0-5.0	0.003	2.93	0.001	0.003	-
Indian subcontinent ⁶⁶	Cauveri river sediment	-	-	2.0-4.0	0.003	1.51	0.001	0.003	-
Japan ⁶⁷	Lake Biwa sediment	-	-	198	-	-	-	-	-
Lake Ohakuri, New Zealand ²⁶	Sediment pore water	-	-	0.98-6.43	-	-	-	-	-
Missouri, USA ²³	Bulk soil	-	-	7	(nearer to smelter)	-	-	-	-
Missouri, USA ²³	Limonite separates	len	-	4.4-84	-	1.8-7.6	-	-	-
Monchegorsk, Russia ⁶⁸	Top soil (0-5 cm) (near smelter)	-	Ni	11-60	0.2	1.6	0.003	0.004	0.1
New Brunswick, Canada ⁵³	Sediment	-	Ag, Cu, Pb, Zn	42	0.02	3.9	0.01	0.02	3.2
Northern Tyrrhenian Sea ⁶⁹	Silty clays	ba, sp,	Co, Cu, Ni, Pb, Zn	24-38	0.004	-	0.007	0.02	-
Northern Tyrrhenian Sea ⁶⁹	Clayey silts	-	-	23-25	0.004	-	0.006	0.01	-
Orissa, India ⁷⁰	Mahanadi river sediment	-	Cu, Fe, Pb	-4	-	-	-	-	-
Orissa, India ⁷⁰	Brahmani river sediment	-	Cu, Fe, Pb	-8	-	-	-	-	-
Orissa, India ⁷⁰	Baitarani river sediment	-	Cu, Fe, Pb	-7	-	-	-	-	-
Qubec & Ontario, Canada ²⁴	Lake sediments	-	-	0.67-40	-	0.2-11.1	-	-	-
Smith County, Tennessee ⁶²	Chattanooga raw shale	oil shale	-	11	0.01	0.7	0.002	0.1	0.8
South Carolina, USA ⁷¹	Coal fly ash tank water	-	-	1300-48900	2.6-3.0	22.0-34.5	-	8.0-12.0	-
South Carolina, USA ⁷¹	Suspended solids	-	-	23-870	0.01-0.02	21.1-39.5	-	0.02-	-
Sudbury, Ontario ²⁷	Lake (suspended particles)	-	-	6.5-38	(nearer to smelter)	-	-	-	-
Sudbury, Ontario ²⁷	Kelly Lake sediment (max)	-	-	650	-	-	-	0.05	-

asp, arsenopyrite; au, placer gold; ba, barite; ccp, chalcopyrite; col, cobaltite; gn, galena; len, lemonite; po, pyrrhotite; py, pyrite; sel, selenite; sp, spalerite; P, present study.

ciation with limonite in the alluvial sediments. Variations in redox conditions modify the concentration of dissolved species and may effect the amount of adsorbed arsenic²⁴. As redox conditions become increasingly reducing at depths, the ferric iron is reduced to ferrous iron, resulting in mobilization of some of the adsorbed arsenic. Under prolonged reducing conditions, essentially all of the adsorbed arsenic may be reduced to As³⁺ (ref. 23). In this connection it is noteworthy that *Anabaena oscillaroides*, a bacteria isolated from the Waikato river is capable of reducing As⁵⁺ to As³⁺ which is more toxic and about 25 times more soluble than the former²⁵. Thus post-depositional changes in redox potentials and pH can potentially modify the movement of As to and from dissolved and solid phases with speciation of As playing an important role in its upward or downward mobility with depth either due to simple inorganic reactions or biochemical reactions. Since some of the sulphide mines are at greater depths, the transport of As in the aquatic environment in the mining areas is very important.

In order to predict the distribution of arsenic between dissolved and particulate phases in a wide range of ecosystems shown in second part of the Table 3, the key role played by Fe oxyhydroxides in the chemistry of dissolved arsenic concentration in oxic aquatic environments must be considered. Many studies have reported the distribution of arsenic among the Fe oxyhydroxides of oxic sediments and the associated interstitial and overlying waters in a variety of lakes^{24,26,27}. Distributions of dissolved As and Fe concentrations suggest a close association of both elements in a dissolution-diffusion-precipitation cycle for Fe and adsorbed As²⁴. Aquatic plants play a key role in remobilizing As at depths which can be illustrated by the following observations.

To gain a better understanding of the impact of rhizosphere/burrow oxidation by wetland plants and burrowing invertebrates on the biogeochemistry of metals and metalloids in the ecosystem, it was hypothesized^{28,29} that salt marsh plants and burrowing invertebrates could accumulate Fe, Zn and As through oxidation of the rhizosphere/burrow wall. The mechanism behind the accumulation would be as follows: Oxidation of ferrous iron to its ferric form would lead to precipitation of iron oxyhydroxides in the rhizosphere (iron plaque), which in turn would lead to a decreasing concentration gradient of dissolved iron oxyhydroxides in the rhizosphere, that in turn would lead to a decreasing concentration gradient of dissolved iron towards the plant roots. The iron oxyhydroxides in turn would bind arsenic and zinc, again creating a decreasing concentration gradient of both elements towards the roots. These gradients would lead to the diffusion of iron, arsenic and zinc in the direction of the roots. Assuming that uptake of the elements by the root is slower than the supply through

diffusion, an increase in concentration of all three elements would be expected to occur in the solid phase of the rhizosphere²⁸. Therefore arsenic contained in sediments or in sulphide ores/concentrates can be released, either as soluble (As³⁺) or insoluble (As⁵⁺) species. In either case, it will be a poison released from the medium and pose a threat to the environment. While the As levels in the groundwater in West Bengal, Bangladesh, outer Mongolia and Japan³⁰ have been widely reported for a long time, it is surprising that As has not been analysed in the sulphide ores of Singbhum base-metal belt that is in the hinterland of the As-contaminated area in India. Along with Cu, Pb and Zn, As may also be amenable to efficient bioleaching processes from sulphide ore deposits³¹. With regard to the As problem in Bangladesh and West Bengal, Fe-oxyhydroxides are reported³² to be the carriers of As in the Gangetic alluvium, and upon redox changes associated with water-table fluctuation in response to withdrawal of water through millions of tube-wells in the area, they release As to the water in soluble form. Also, there are weathered base metal deposits underlying the Gangetic basin³³ which could be the source of As as observed for the sulphide deposit in the present study. Arsenic-bearing phosphatic fertilizers also could be an additional source of As³³. The chemical speciation of arsenic as well as the Eh values of affected groundwater in Bangladesh indicate the tendency towards reducing environment³⁴. Furthermore, comparison between total arsenic and iron content in groundwater, aquifer soil, suspended river sediment, bank sediment and surface soil does not indicate strong correlation³⁴, thereby contradicting earlier work³² in Fe-As relationship. Thus mobilization of As needs to be carefully studied in future from the source to the aquifer region. However, in the Khetri-Zawar region, the As problem may not be associated with movement in aquifers like in the Bengal delta area and thus different mechanisms of transport of As in the aquatic system may operate in various regions and no single mechanism can account for all observations.

Thus our observation indicates that from the sulphide ores the mine water leaches out As under redox and acidic conditions and further As concentration takes place in the metal sulphide beneficiation activities. Thus no specific As-enriched mineral such as arsenopyrite is required in the neighbouring lithology but all sulphide mining and smelter sites are potential locations of future As-contaminated areas and should not await an episodic event to unleash an environmental disaster. Arsenic studies should therefore precede mining/smelter operations as a precaution in all sulphide-related mineral resources exploitation.

I. Azcue, J. M. and Nriagu, J. O., *Arsenic in the Environment*, John Wiley, New York, 1994, pp. 1-17.

2. WHO Guidelines for Drinking Water Quality, Recommendations, 2nd edn, World Health Organisation, Geneva, 1993, vol. 1, p. 41.
3. US Environmental Protection Agency (US-EPA), *Quality Criteria for Water*, US-EPA, Washington DC, 1976.
4. Mandal, B. K., Roy Chowdhury, T., Samanta, G., Basu, G. K., Chowdhury, P. P., Chanda, C. R., Lodh, D., Karan, N. K., Dhar, R. K., Tamili, D. K., Das, D., Saha, K. C. and Chakraborti, D., *Curr. Sci.*, 1996, **70**, 976-986.
5. Mandal, B. K., Roy Chowdhury, T., Samanta, G., Basu, G. K., Chowdhury, P. P., Chanda, C. R., Lodh, D., Karan, N. K., Dhar, R. K., Tamili, D. K., Das, D., Saha, K. C. and Chakraborti, D., *Curr. Sci.*, 1997, **72**, 114-117.
6. Bagla, P. and Kaiser, J., *Science*, 1996, **274**, 174-175.
7. Saha, D. P. and Subramanian, K. S., *Science*, 1996, **274**, 1285-1289.
8. Dhar, R. K., Biswas, B. K., Samanta, G., Mandal, B. K., Chakraborti, D., Roy, S., Jafar, A., Islam, A., Ara, G., Kabir, S., Khan, A. W., Ahmed, S. A. and Hadi, S. A., *Curr. Sci.*, 1997, **73**, 48-59.
9. Biswas, B. K., Dhar, R. K., Samanta, G., Mandal, B. K., Chakraborti, D., Faruk, I., Islam, K. S., Chowdhury, Md. M., Islam, A. and Roy, S., *Curr. Sci.*, 1998, **74**, 134-145.
10. Mushtaque, A., Chowdhury, R. and Jakariya, M., *Science*, 1999, **284**, 1621.
11. Peters, S. C., Blum, J. D., Klaue, B. and Karakas, M. R., *Environ. Sci. Technol.*, 1999, **33**, 1328-1333.
12. Laznicka, L., *Econ. Geol.*, 1999, **94**, 455-474.
13. Onken, B. M. and Hossner, L. R., *Soil Sci. Soc. Am. J.*, 1996, **60**, 1385-1392.
14. Klein, C. and Hurlbut, C. S., *Manual of Mineralogy*, John Wiley, New York, 1985, 20th edn.
15. Taylor, S. R. and McLennan, S. M. *The Continental Crust: Its Composition and Evolution*, Blackwell Scientific Publications, Boston, 1985.
16. Baker, L. A., Aureshi, T. M. and Wyman, M. M., *Water Resour. Res.*, 1998, **34**, 1543-1552.
17. Wilkie, J. A. and Hering, J. G., *Environ. Sci. Technol.*, 1998, **32**, 657-662.
18. Korte, N., *Environ. Geol. Water Sci.*, 1991, **18**, 137-141.
19. Peryea, F. J., *Soil Sci. Soc. Am. J.*, 1991, **55**, 1301-1306.
20. Mukherjee, A. D., Samhita Glahiri and Bhattacharya, H. N., *J. Geol. Soc. India*, 1991, **37**, 132-135.
21. GBC 902 and HG 900, *Operation Manual for the Hydride Generator*, 1986, pp. 1-12.
22. Mok, W. M., Riley, J. A. and Wai, C. M., *Water Res.*, 1988, **22**, 769-774.
23. Korte, N., *Environ. Geol. Water Sci.*, 1991, **18**, 137-141.
24. Belzile, N. and Tessier, A., *Geochim. Cosmochim. Acta*, 1990, **54**, 103-109.
25. McLaren, S. J. and Kim, N. D., *Environ. Pollut.*, 1995, **90**, 67-73.
26. Aggett, J. and Kriegman, M. R., *Water Res.*, 1988, **22**, 407-411.
27. Nariagu, J. O., *Geochim. Cosmochim. Acta*, 1983, **47**, 1523-1526.
28. Otte, M. L., Kearns, C. C. and Doyle, M. O., *Bull. Environ. Contam. Toxicol.*, 1995, **55**, 154-161.
29. Doyle, M.O. and Otte, M. L., *Environ. Pollut.*, 1997, **96**, 1-11.
30. Guo Xiao-Juan, Report, News Letter 2, Asia Arsenic Network (AAN), 1999.
31. Natarajan, K. A., in *Biogeochemistry of Rivers in Tropical South and Southeast Asia* (eds Ittekkot, V., Subramanian, V. and Annadurai, S.), Heft 82, SCOPE Sonderband, Mitt. Geol.-Paläont. Inst., Univ. Hamburg, 1999, pp. 55-72.
32. Nickson, R., McArthur, J., Burgess, W., Ahmed, K. M., Ravenscroft, P. and Rahman, M., *Nature*, 1998, **395**, 338.
33. Acharyya, S. K., Chakraborty, P., Lahiri, S., Raymahashay, B. C., Guha, S. and Bhowmik, A., *Nature*, 1999, **401**, 545-547.
34. Safiullah, S., Khanal, D. P., Tareq, S. M. T. and Khan, M. M. K., in *Biogeochemistry of Rivers in Tropical South and Southeast Asia* (eds Ittekkot, V., Subramanian, V. and Annadurai, S.), Heft 82, SCOPE Sonderband, Mitt. Geol.-Paläont. Inst., Univ. Hamburg, 1999, pp. 125-130.
35. Konhauser, K. O., Powell, M. A., Fyfe, W. S., Longstaffe, F. J. and Tripathy, S., *Environ. Geol.*, 1997, **29**, 132-141.
36. Boyle, D. R., Turner, R. J. W. and Hall, G. E. M., *Environ. Geochem. Health*, 1998, **20**, 199-212.
37. Mok, W. M., Riley, J. A. and Wai, C. M., *Water Res.*, 1988, **22**, 769-774.
38. Johnson, C. A. and Thornton, I., *Water Res.*, 1987, **21**, 359-365.
39. Periakali, P., Research proposal submitted to the Department of Science and Technology, New Delhi, 1998.
40. Wilson, F. H. and Hawkins, D. B., *Environ. Geol.*, 1978, **2**, 195-202.
41. Cidu, R., Caboi, R., Fanfani, L. and Frau, F., *Environ. Geol.*, 1997, **30**, 231-237.
42. Neal, C., Simith, C. J., Jeffery, H. A., Jarvie, H. P. and Robson, A. J., *J. Hydrol.*, 1996, **182**, 37-64.
43. Sonderegger, J. L. and Ohguchi, T., *Environ. Geol. Water Sci.*, 1988, **11**, 153-161.
44. Rosner, U., *Environ. Geol.*, 1998, **33**, 224-230.
45. Maher, W. A., *Water Res.*, 1985, **19**, 933-934.
46. Kwong, Y. T. J., Roots, C. F., Roach, P. and Kettley, W., *Environ. Geol.*, 1997, **30**, 98-107.
47. Boulet, M. P. and Larocque, A. C. L., *Environ. Geol.*, 1998, **33**, 130-142.
48. Devaraju, T. C. and Alapieti, T. T., *J. Geol. Soc. India*, 1997, **49**, 597-598.
49. David Banks, Paul L. Younger, Rolf-Tore Arnesen, Egil R. Iversen and Sheila, B. B., *Environ. Geol.*, 1997, **32**, 157-174.
50. Holmstrom, H., Ljungberg, J. and Ohlander, B., *Environ. Geol.*, 1999, **37**, 267-280.
51. Pichler, T., Giggenbach, W. F., McInnes, B. I. A., Buhl, D. and Duck, B., *Econ. Geol.*, 1999, **94**, 281-288.
52. Manz, M. and Castro, L. J., *Environ. Pollut.*, 1997, **98**, 7-13.
53. Lentz, D. R., *Econ. Geol.*, 1999, **94**, 57-86.
54. Walder, I. F. and Chavez, W. X. Jr., *Environ. Geol.*, 1995, **26**, 1-18.
55. Muller, G. A., *Econ. Geol.*, 1997, **92**, 181-209.
56. Dill, H. G., Perfold, Z. and Riera, K. C., *Econ. Geol.*, 1997, **92**, 623-632.
57. Benvenuti, B., Mascaro, I., Corsini, F., Lattanzi, P., Parrini, P. and Tanelli, G., *Environ. Geol.*, 1997, **30**, 238-243.
58. Simon, G., Kesler, S. E. and Chryssoulis, S., *Econ. Geol.*, 1999, **94**, 405-422.
59. Datta, D. K. and Subramanian, V., *Environ. Geol.*, 1997, **30**, 181-188.
60. Datta, D. K. and Subramanian, V., *Environ. Geol.*, 1998, **36**, 93-101.
61. Mok, W. M. and Wai, C. M., *Water Res.*, 1989, **23**, 7-13.
62. Sullivan, P. J. and Yelton, J. L., *Environ. Geol. Water Sci.*, 1988, **12**, 181-186.
63. Klassen, R. A., *Environ. Geol.*, 1998, **33**, 154-169.
64. Leoni, L. and Sartori, F., *Environ. Geol.*, 1997, **32**, 83-91.
65. Pirrie, D., Camm, G. S., Sear, L. G. and Hughes, S. H., *Environ. Geol.*, 1997, **29**, 58-65.
66. Subramanian, V., Van Grieken, R. and Van T'Dack, L., *J. Geol. Soc. India*, 1987, **30**, 217-226.
67. Takamatsu, T., Kawashima, M. and Koyama, M., *Water Res.*, 1985, **19**, 1029-1032.
68. Reimann, C., Boyd, R., Caritat, P. de., Halleraker, J. H., Kashulina, G., Niskavaara, H. and Bogatyrev, I., *Environ. Pollut.*, 1997, **95**, 45-56.

69. Leoni, L., Sartori, F., Saitta, M., Damiani, V., Ferretti, O. and Viel, M., *Environ. Geol. Water Sci.*, 1971, **17**, 23–46.
70. Konhauser, K. O., Powell, M. A., Fyfe, W. S., Longstaffe, F. J. and Tripathy, S., *J. Hydrol.*, 1997, **193**, 258–269.
71. Newman, M. C., Alberts, J. J. and Greenhut, V. A., *Water Res.*, 1985, **19**, 1157–1165.

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Antimicrobial activity of artemisinin and its precursors

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Artemisinic acid and arteannuin B are biogenetic precursors of artemisinin, an important antimalarial produced by the herb *Artemisia annua*. These compounds have been screened for antimicrobial activity against a range of organisms. All the three compounds are active against different bacteria and certain fungal species.

ARTEMISININ and its biosynthetic precursors, arteannuin B and artemisinic acid are important secondary metabolites produced by a Chinese medicinal plant *Artemisia annua*. Artemisinin, a sesquiterpene lactone endoperoxide, is a promising antimalarial drug. The extracts of the plant have been used in traditional Chinese medicine for treatment of chills, fever and malaria¹. Artemisinin and its derivatives have been reported to cause inhibition of plaque formation in *Toxoplasma gondii* and are active against *Leishmania major*^{2,3}. They are also active against *Schistosoma mansoni* and *S. japonicum* both *in vitro* and *in vivo*⁴. At higher concentrations, artemisinin-related endoperoxides have been found to have cytotoxicity to Ehrlich ascites tumour cells, human HeLa cells and murine bone marrow using a CFU-GM assay^{5,6}. This drug has the potential to be used as a herbicide and algicide at very low concentrations. It has significant cytotoxic activity against P-388, A-549, HT-29, MCF-7, KB tumour cell lines and EN2 tumour cells^{7,8}. Artemisinin was also found to be effective against avian coccidiosis when given in low concentrations as a food additive. It has been reported to cause a significant reduction in the lesions caused by the parasite *Eimeria tenella*^{9,10}. Artemisinic acid is a biogenetic precursor of artemisinin. Its derivatives are re-

ported to be cytotoxic *in vitro* to human hepatoma cell line SMMC-7721, human embryonic lung cell line WI-38, human gastric cancer line SCG-7901 and murine leukaemia cell line P-388 (ref. 11). Arteannuin B is considered to be an intermediate in the bioconversion of artemisinic acid to artemisinin. Arteannuin B has an unusual α -methylene- γ -lactone, being transfused via a tertiary hydroxyl group at the *sis* decalin ring fusion¹². It has attracted much interest due to its potential antitumour activity¹³.

There have been some reports on the antimicrobial activities of some constituents of other *Artemisia* species. However, no detailed studies have been carried out on the antimicrobial properties of the extracts of *A. annua* in particular artemisinin, arteannuin B and artemisinic acid. The present paper reports a study on the antimicrobial properties of artemisinin and its biosynthetic precursors.

Artemisinin was procured from Sigma Chemical Company, St. Louis, MO, USA. Pure samples of arteannuin B and artemisinic acid were a kind gift from Hala N. Elsholy, University of Mississippi, USA and Jorge Ferreira, AgrEvo Research Centre, Pikeville, USA. The bacteria and fungi (listed in Table 1) used for testing for the activity of artemisinin and its precursors were grown on suitable culture media and subsequently used as test organisms. The antimicrobial activity of the compounds was evaluated using the cup-plate method. The compounds were dissolved in 100 μ l of dimethylformamide (DMF) and loaded in wells (8 mm diameter) on suitable agar plates. All the experiments were carried out in duplicates and repeated thrice. The medium used for all the assays was nutrient agar in the case of most bacterial cultures and potato dextrose agar in the case of fungi. *E. coli* M200 ATCC 14169, *Streptococcus faecalis* ATCC 8043, and *Salmonella abony* NCTC 6017 were however assayed on media recommended by the Indian Pharmacopoeia (B₁₂ culture agar HM Code M-185, folic acid inoculum medium and xylose lysine deoxycholate agar HM code M-031, respectively). All the procedures carried out were in accordance with the British Pharmacopoeia, 1993 and the Indian Pharmacopoeia, 1996. The bacterial and fungal test organisms were grown at 37, 30 or 25°C as the case may be for individual cultures. The bacterial cultures (2% v/v inoculum at 25% transmission at 530 nm) were mixed with molten agar medium and used for preparation of plates. Different concentrations of artemisinin, arteannuin B and artemisinic acid (ranging from 250 ng to 500 μ g) dissolved in DMF were added into the wells of the plates and incubated for 48 h at 37°C in the case of pathogenic bacteria, 30°C in the case of *Bacillus* and *Agrobacterium* species and 25°C in the case of fungal cultures. After 48 h of incubation the zones of inhibition were measured. In order to assess the potency of these compounds, the zones of inhibition obtained with differ-

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